UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

August 13, 1998

Note to the Reader:

The attached draft report is a draft report of the Science Advisory Board (SAB). The draft is still undergoing final internal SAB review, however, in its present form, it represents the consensus position of the panel involved in the review. Once approved as final, the report will be transmitted to the EPA Administrator and will become available to the interested public as a final report.

This draft has been released for general information to members of the interested public and to EPA staff. This is consistent with the SAB policy of releasing draft materials only when the Committee involved is comfortable that the document is sufficiently complete to provide useful information to the reader. The reader should remember that this is an unapproved working draft and that the document should not be used to represent official EPA or SAB views or advice. Draft documents at this stage of the process often undergo significant revisions before the final version is approved and published.

The SAB is not soliciting comments on the advice contained herein. However, as a courtesy to the EPA Program Office which is the subject of the SAB review, we have asked them to respond to the issues listed below. Consistent with SAB policy on this matter, the SAB is not obligated to address any responses which it receives.

1. Has the Committee adequately responded to the questions posed in the Charge?
2. Are any statements or responses made in the draft unclear?
3. Are there any technical errors?

For further information or to respond to the questions above, please contact:

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&EPA

AN SAB REPORT: REVIEW OF THE HEALTH RISK ASSESSMENT OF 1,3-BUTADIENE

REVIEW OF THE OFFICE OF RESEARCH AND DEVELOPMENT'S DRAFT HEALTH RISK ASSESSMENT OF 1,3-BUTADIENE PREPARED BY THE ENVIRONMENTAL HEALTH COMMITTE (EHC)

EPA-SAB-EHC-98-

Honorable Carol M. Browner

Administrator

U.S. Environmental Protection Agency

401 M Street, S.W.

Washington, DC 20460

Subject: Review of the Health Risk Assessment of 1,3-Butadiene

Dear Ms. Browner:

1   At the request of the Office of Research and Development (ORD), the Environmental

2   Health Committee (EHC) of the Environmental Protection Agency's Science Advisory Board

3   (SAB) reviewed the Agency's Health Risk Assessment of 1,3-butadiene. The Committee met on

4   April 30 and May 1, 1998 at the EPA's Waterside Mall Complex in Washington DC. The EHC

5   approved the Committee's report on August 11, 1998 and the SAB's Executive Committee

6   approved this report on _____.

7

8   The Health Risk Assessment of 1,3-butadiene which was reviewed by the Committee was

9   developed by the Office of Research and Development. ORD published its first risk assessment of

10   1,3-butadiene in 1985. The first document covered cancer and mutagenicity and was prepared in

11   response to a request from the Office of Air Quality Planning and Standards to support the

1 classification of 1,3-butadiene as a Hazardous Air Pollutant. The recent draft 1,3-butadiene

2 document was written in response to a request from the Agency's Office of Mobile Sources. The

3 Agency plans to use the final document to support a future Air Toxics Rule. The recent draft

4 1,3-butadiene document (the review document) focuses on mutagenicity, carcinogenicity, and

5 reproductive/developmental effects. The 1,3-butadiene document which was reviewed by the

6 Committee presents the Agency's first benchmark dose analysis for reproductive/developmental

7 factors. The review document includes many new studies which have been published since 1985.

8 The EPA concludes, in its draft health risk assessment of 1,3-butadiene, that this new information

9 has changed the weight of evidence for cancer. In addition, there are exposure data available in an

10 occupational study which are used to derive the cancer slope factor. The review document is not

11 intended to be a comprehensive health assessment. For example, it contains an overview of the

12 ambient exposure and exposure of populations adjacent to emissions sources, without any actual

13 exposure assessment as such.

14

15   In addition to a general review of the document by the Committee, the Office of Research

16 and Development specifically requested that the EHC provide comment on each of the following

17 aspects of the document:

18

19 a) Review the health risk assessment for technical quality, comprehensiveness and clarity

20   (Address each chapter, but with specific reference to Charges b, c, and d).

21

22 b) Does the science support the classification of "known" human carcinogen?

23

24 c) Are the approaches taken to characterize plausible cancer risks reasonable given the

25   science?

26

27 d) Are the conclusions and quantitative estimations for reproductive/developmental

1   effects adequately supported?

2

3   The EHC recognizes that preparing a health risk assessment of 1,3-butadiene was a difficult and

4   complicated task given the large amount of information on 1,3-butadiene toxicity, epidemiology,

5   and mechanisms available, with new and relevant information being made on a continual basis.

6   Furthermore, there has been a significant body of literature published since the last carcinogen

7   assessment for 1,3-butadiene which was conducted by the EPA in 1985.

8

9   The Agency selected a cutoff date for the inclusion of new information in the revised, draft

10   document of January 31, 1997. However, a significant amount of new and important information

11   has been developed since then and is pertinent to a health risk assessment of

12   1,3-butadiene. The Committee felt that the report should reflect the most current research data,

13   including the recent evaluations by the International Agency for Research on Cancer, and if

14   finalized soon, the evaluation of Health Canada (IARC, 1998)(Health Canada, 1997). Therefore,

15   with respect to the first charge question on the technical quality, comprehensiveness and clarity of

16   the document, the EHC found that the quality and comprehensiveness would be greatly improved

17   by including research data published in the peer-reviewed literature since the cutoff date of

18   January 31, 1997. The EHC notes that important research such as the Delzell et al. (1995)

19   exposure reestimation and the pharmacokinetic modeling studies is ongoing. To improve the

20   clarity of the document, the Committee recommended several editorial changes, such as the

21   inclusion of summary tables in some of the chapters.

22

23   The majority of the Environmental Health Committee did not classify 1,3-butadiene as a

24   known human carcinogen due to the lack of consistency between exposure and leukemia or

25   lymphosarcoma data when the styrene-butadiene rubber (SBR) and monomer worker studies were

26   considered in total. The majority opined that 1,3-butadiene should be classified as a probable

27   human carcinogen whereas a minority felt that the science supported the classification

1   of 1,3-butadiene as a known human carcinogen. The vast majority of the panel felt that the SBR

2   process supported a classification as a known carcinogen. The majority opinion was based on

3   several lines of evidence: a) There was only one study on workers in the monomer process that

4   showed an excess of lymphosarcoma but this study has not been replicated. (One would like to

5   see at least a second independent confirmatory study before affirming that there is "sufficient

6   evidence of human carcinogenicity" regarding butadiene and leukemia. Instead a fairly large and

7   reasonable second study shows no leukemia excess and two smaller ones found no evidence of

8   leukemia risk); b) In the monomer study, there was no evidence of an exposure response

9   relationship for lymphosarcoma nor was there evidence that those workers with longer-term

10   exposure had a higher risk of lymphosarcoma; and c) A large study of the workers in the

11   styrene-butadiene rubber (SBR) industry showed an excess of leukemia. However, since these

12   workers were exposed to several different chemicals, the cancer excess could not be attributed

13   solely to 1,3-butadiene. Furthermore, the findings from the reevaluation of the exposure estimates

14   in the Delzell et al. (1995) study may impact the risk assessment. The majority of the Committee

15   felt that the finding of 'known human carcinogen' should solely be based on observational studies

16   in humans, without regard to mechanistic or other information. Others on the Committee felt that

17   butadiene should be identified as a 'known human carcinogen' because the cumulative evidence,

18   from epidemiology, animal cancer bioassays, and mechanistic studies should be used as the basis

19   for the judgment.

20

21   The Committee found the approaches taken to characterize plausible cancer risks to be

22   reasonable but points out specific data that may have been misinterpreted by the Agency. In

23   particular, the discussion of metabolism and toxicokinetics failed to address critical differences in

24   metabolites of butadiene in different species accounting for differences in tumor susceptibility in

25   different species. Inclusion of a discussion of state-of-the-art models for butadiene kinetics would

26   significantly strengthen the scientific quality of the document. Included in this discussion should

27   be the strengths and weaknesses of the available models for risk prediction. This fuller

1   discussion should replace the simple statements made about the inadequacy of the available

2   models.

3

4   The Committee commends the Agency for looking at new approaches, such as the

5   benchmark dose procedure, to improve quantitative assessment of non cancer endpoints.

6   However, the Committee has submitted suggestions on how to further improve these approaches

7   and how to make these new approaches more clear, accurate and concise, including the following

8   recommendations:

9   a)   There was an apparent mathematical error in the calculation of the benchmark

10   concentration for reproductive and developmental effects which must be

11   addressed. All calculations should be easy to follow, and of course, carefully

12   proofed;

13   b)   There are new dominant lethal studies, which are not included in the risk

14   assessment, that failed to replicate earlier findings;

15   c)   A variety of viewpoints were expressed within the Committee over the extent to

16   which toxicokinetic analyses should be incorporated into the assessment. At a

17   minimum the different toxicokinetic hypotheses and the hypothesized role of the

18   various metabolites should be discussed, at least qualitatively, with an indication of

19   the degree to which the assessment would be impacted if some of the hypotheses

20   were later proven true.

21   d)   Inadequate justification is given for the application of the additional safety factor

22   for the benchmark dose. Some of the Committee members could not understand

23   the rationale for its inclusion, and

24   e)   The rationale for the selection of the toxic non-cancer endpoint that is utilized in

25   the derivation of the RfC is very important and should be more explicitly

26   explained.

27

1   The Committee appreciates the opportunity to review the draft Health Risk Assessment of

2   1,3-butadiene and looks forward to receiving a written response from the Director of the Office of

3   Research and Development, National Center for Environmental Assessment.

4

5

6   Sincerely,

7

8   Dr. Joan M. Daisey, Chair

9   Executive Committee

10

11   Dr. Emil A. Pfitzer, Chair

12   Environmental Health Committee

13

14   Mark J. Utell, Co-Chair,

15   Environmental Health Committee

16

1 # NOTICE

2

3   This report has been written as part of the activities of the Science Advisory Board, a

4 public advisory group providing extramural scientific information and advice to the Administrator

5 and other officials of the Environmental Protection Agency. The Board is structured to provide

6 balanced, expert assessment of scientific matters related to problems facing the Agency. This

7 report has not been reviewed for approval by the Agency and, hence, the contents of this report

8 do not necessarily represent the views and policies of the Environmental Protection Agency, nor

9 of other agencies in the Executive Branch of the Federal government, nor does mention of trade

10 names or commercial products constitute a recommendation for use.

11

ABSTRACT

The Environmental Health Committee (EHC) reviewed the EPA's updated, draft health risk assessment of 1,3-butadiene. The Committee acknowledged that this is an extremely difficult task given the large amount of information on 1,3-butadiene toxicity, epidemiology, and mechanisms, with new information being introduced on a continual basis.

The Agency selected a cutoff date for the inclusion of new information in the revised, draft document of January 31, 1997. However, a significant amount of new and important information has been developed since then and is pertinent to a health risk assessment of 1,3-butadiene. The Committee felt that the report should reflect the most current research data, including the recent evaluations by the International Agency for Research on Cancer, and if finalized soon, the evaluation of Health Canada. The EHC notes that important research such as the Delzell et al. (1995) exposure reestimation and the pharmacokinetic modeling studies is ongoing. To improve the clarity of the document, the Committee recommended several editorial changes, such as the inclusion of summary tables in some of the chapters.

The majority of the Environmental Health Committee did not classify 1,3-butadiene as a known human carcinogen due to the lack of consistency between exposure and leukemia or lymphosarcoma data when the styrene-butadiene rubber (SBR) and monomer worker studies were considered in total. The majority opined that 1,3-butadiene should be classified as a probable human carcinogen where as a minority felt that the science supported the classification of 1,3-butadiene as a known human carcinogen. The vast majority of the panel felt that the SBR process supported classification as a known carcinogen. The majority opinion was based on several lines of evidence: a) There was only one study of workers in the monomer process that showed an excess of lymphosarcoma but this study has not been replicated; b) In the monomer study, there was no evidence of an exposure response relationship for lymphosarcoma nor was

1   there evidence that those workers with longer-term exposure had a higher risk of lymphosarcoma;

2   and c) A large study of the workers in the styrene-butadiene rubber (SBR) industry showed an

3   excess of leukemia. However, since these workers were exposed to several different chemicals,

4   the cancer excess could not be attributed solely to 1,3-butadiene.

5

6   The Committee found the approaches taken to characterize plausible cancer risks to be

7   reasonable but points out specific data that may have been misinterpreted by the Agency. In

8   particular, the discussion of metabolism and toxicokinetics failed to address critical differences   in

9   metabolites of butadiene in different species accounting for differences in tumor susceptibility.

10   Inclusion of a discussion of state-of-the-art models for butadiene kinetics would significantly

11   strengthen the scientific quality of the document. Included in this discussion should be the   strengths

12   and weaknesses of the available models for risk prediction. This fuller discussion   should replace

13   the simple statements made about the inadequacy of the available models.

14

15   The Committee supported the use of the benchmark dose procedure in developing

16   Reference levels. The Committee submitted suggestions on how to further improve the

17   approaches for quantitative assessment of non-cancer endpoints, such as the

18   reproductive/developmental effects, and how to make the approach more clear, accurate and

19   consistent. All calculations should be easy to follow, and of course, carefully proofed. Greater

20   explanation is needed of the safety factors applied to the benchmark, and of the newly proposed

21   models, especially those modeling time to impact. New dominant lethal studies, not included in

22   the risk assessment should be evaluated, especially in light of apparent inconsistency with the

23   earlier findings. The Agency should consider a quantitative assessment based on the heritable

24   translocation endpoint. Also, the EHC recommends that the Agency explain, in more detail, the

25   rationale for the selection of the toxic non-cancer endpoint that is utilized in the derivation of the

26   RfC.

27

1   **Keywords**:   1,3-butadiene, EPA's proposed Cancer Risk Assessment Guidelines, known human

2   carcinogen, probable human carcinogen, lymphosarcoma, leukemia,

3   reproductive/developmental effects, pharmacokinetics, risk assessment

4

1

U.S. ENVIRONMENTAL PROTECTION AGENCY
SCIENCE ADVISORY BOARD
ENVIRONMENTAL HEALTH COMMITTEE
1,3-Butadiene Panel

6

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DRAFT #4   **- DRAFT DOCUMENT-**
                               - DO NOT CITE OR QUOTE -

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1. EXECUTIVE SUMMARY

The EPA Office of Research and Development prepared the draft *Health Risk Assessment of 1,3-Butadiene* (USEPA, 1998a) in response to a request from the Office of Mobile Sources (OMS). The document was requested by OMS in order to support a future Air Toxics Rule. The review document is not intended to be a comprehensive health assessment and therefore, does not contain any actual exposure assessment. The document focuses on mutagenicity, carcinogenicity, and reproductive/developmental effects and presents the Agency's first benchmark dose analysis for reproductive/developmental effects. In its document, the Agency has found that the new studies which have been published since 1985 change the weight of evidence for cancer. Based on the weight of overall evidence from human, animal, and mutagenicity studies, the Agency concludes that 1,3-butadiene is a known human carcinogen.

On April 30 and May 1, 1998, the Environmental Health Committee met at the EPA's Waterside Mall complex in Washington, DC to review the Agency's draft *Health Risk Assessment of 1,3-Butadiene*. In addition to a general review of the document by the Committee, the Office of Research and Development specifically requested that the EHC provide comment on each of the following aspects of the document:

a)   Review the health risk assessment for technical quality, comprehensiveness and clarity (Address each chapter, but with specific reference to Charges b, c, and d).

b)   Does the science support the classification of "known" human carcinogen?

c)   Are the approaches taken to characterize plausible cancer risks reasonable given the science?

1

2   d)   Are the conclusions and quantitative estimations for reproductive/developmental

3   effects adequately supported?

4

5   The Committee acknowledges that this is an extremely difficult task given the large

6 amount of information on 1,3-butadiene toxicity, epidemiology, and mechanism available with

7 new information being made on a continual basis.

8

9   The Agency selected a cutoff date for the inclusion of new information in the revised, draft

10 document of January 31, 1997. However, a significant amount of new and important information

11 has been developed since then and is pertinent to a health risk assessment of 1,3-butadiene. The

12 Committee felt that the report should reflect the most current research data, including the recent

13 evaluations by the International Agency for Research on Cancer and on ongoing evaluation in

14 Canada (Health Canada, 1997)(IARC, 1998). Therefore, with respect to the first charge question

15 on the technical quality, comprehensiveness and clarity of the document, the EHC found that the

16 quality and comprehensiveness would be greatly improved by including research data published in

17 the peer-reviewed literature since the cutoff date of January 31, 1997. The EHC notes that

18 important research such as the Delzell et al. (1995) exposure reestimation and the

19 pharmacokinetic modeling studies is ongoing. To improve the clarity of the document, the

20 Committee recommended several editorial changes, such as the inclusion of summary tables in

21 some of the chapters.

22

23   The majority of the Environmental Health Committee did not classify 1,3-butadiene as a

24 known human carcinogen due to the lack of consistency between exposure and leukemia or

25 lymphosarcoma data when the styrene-butadiene rubber (SBR) and monomer worker studies were

26 considered in total. The majority opined that 1,3-butadiene should be classified as a probable

27 human carcinogen where as a minority felt that the science supported the classification of

1   1,3-butadiene as a known human carcinogen. The vast majority of the panel felt that the SBR

2   process supported a classification as a known carcinogen. The majority opinion was based on

3   several lines of evidence: a) There was only one study on workers in the monomer process that

4   showed an excess of lymphosarcoma but this study has not been replicated . (One would like to

5   see at least a second independent confirmatory study before affirming that there is "sufficient

6   evidence of human carcinogenicity" regarding butadiene and leukemia. Instead a fairly large and

7   reasonable second study shows no leukemia excess and two smaller ones found no evidence of

8   leukemia risk); b) In the monomer study, there was no evidence of an exposure response

9   relationship for lymphosarcoma nor was there evidence that those workers with longer-term

10 exposure had a higher risk of lymphosarcoma; and c) A large study of the workers in the

11 styrene-butadiene rubber (SBR) industry showed an excess of leukemia. However, since these

12 workers were exposed to several different chemicals, the cancer excess could not be attributed

13 solely to 1,3-butadiene. The majority of the Committee felt that the finding of 'known human

14 carcinogen' should solely be based on observational studies in humans, without regard to

15 mechanistic or other information. Others on the Committee felt that butadiene should be identified

16 as a 'known human carcinogen' because the cumulative evidence, from epidemiology, animal

17 cancer bioassays, and mechanistic studies should be used as the basis for the judgment.

18

19   The Committee found the approaches taken to characterize plausible cancer risks to be

20 reasonable but points out specific data that may have been misinterpreted by the Agency. In

21 particular, the discussion of metabolism and toxicokinetics failed to address critical differences in

22 metabolites of butadiene in different species accounting for differences in tumor susceptibility.

23 Inclusion of a discussion of state-of-the-art models for butadiene kinetics would significantly

24 strengthen the scientific quality oft he document. Included in this discussion should be the

25 strengths and weaknesses of the available models for risk prediction. This fuller discussion should

26 replace the simple statements made about the inadequacy of the available models.

27

1   The Committee commends the Agency for looking at new approaches, such as the

2   benchmark dose procedure, to improve quantitative assessment of non cancer endpoints.

3   However, the Committee has submitted suggestions on how to further improve these approaches

4   and how to make these new approaches more clear, accurate and concise, including the following

5   recommendations:

6   a)   There was an apparent mathematical error in the calculation of the benchmark

7   concentration for reproductive and developmental effects which must be

8   addressed. All calculations should be easy to follow, and of course, carefully

9   proofed;

10   b)   There are new dominant lethal studies, which are not included in the risk

11   assessment, that failed to replicate earlier findings;

12   c)   A variety of viewpoints were expressed within the Committee over the extent to

13   which toxicokinetic analyses should be incorporated into the assessment. At a

14   minimum the different toxicokinetic hypotheses and the hypothesized role of the

15   various metabolites should be discussed, at least qualitatively, with an indication of

16   the degree to which the assessment would be impacted if some of the hypotheses

17   were later proven true.

18   d)   Inadequate justification is given for the application of the additional safety factor

19   for the benchmark dose. Some of the Committee members could not understand

20   the rationale for its conclusion, and

21   e)   The rationale for the selection of the toxic non-cancer endpoint that is utilized in

22   the derivation of the RfC is very important and should be more explicitly

23   explained.

2. INTRODUCTION

2.1 Background

      The draft *Health Risk Assessment of 1,3-Butadiene* (USEPA, 1998a) was developed by the EPA Office of Research and Development at the request of the Office of Mobile Sources. This document was requested to support a future Air Toxics Rule. The document was not intended to be a comprehensive health assessment. Consequently, an actual exposure assessment is not included in the document. The draft *Health Risk Assessment of 1,3-Butadiene* (USEPA, 1998a) focuses on carcinogenicity, mutagenicity, and reproductive/developmental effects. The document presents the Agency's first benchmark dose analysis for reproductive/developmental factors.

      The draft document that was reviewed by the Committee updates a previously, published document (USEPA, 1995). In the current draft document, the Agency has included many new studies which have been published since 1985. Based on the weight of overall evidence from human, animal, and mutagenicity studies, the Agency concludes, in the current draft document, that 1,3-butadiene is a known, human carcinogen.

1   **2.2 The Review and Charge**

2

3   On April 30 and May 1, 1998, the EHC Committee met at the EPA's Waterside Mall

4   Complex in Washington, DC to review the Agency's draft *Health Risk Assessment of 1,3-*

5   *Butadiene* document. The Committee was charged to provide comments on each of the following

6   aspects of the document:

7

8   a)   Review the health risk assessment for technical quality, comprehensiveness and

9   clarity (Address each chapter, but with specific reference to Charges b, c, and d).

10

11   b)   Does the science support the classification of "known" human carcinogen?

12

13   c)   Are the approaches taken to characterize plausible cancer risks reasonable given

14   the science?

15

16   d)   Are the conclusions and quantitative estimations for reproductive/developmental

17   effects adequately supported?

18

19

3. RESPONSE TO THE CHARGE

This report captures comments and recommendations made in consensus by the Committee as well as specific technical comments and recommendations provided by individual Committee Members. It was not possible to review and achieve consensus for each and every technical point provided in this Committee report. On major points an attempt has been made to reflect the consensus or range of views on the Committee.

3.1 Technical Quality, Comprehensiveness and Clarity

The Environmental Health Committee was asked to: *review the health risk assessment for technical quality, comprehensiveness, and clarity.* The Committee provided comments and recommendations for each chapter.

3.1.1 Chapter 1 - Introduction

The Introduction presents a clear and comprehensive summary of information on 1,3-butadiene for the period from 1985 until January 31, 1997. It was concluded that apparent differences in assessments by different groups might be explained by the availability of studies at the time of evaluations, different cancer classification systems, and quantitative assessments done for different purposes. Does this mean that they are all equally valid and scientifically defensible? In other words can the statements made in Table 1-1 be restated in comparable terminology to show that they are all compatible? If they are, as implied, then the document should demonstrate this in subsequent chapters. If not, the Introduction should state so, and subsequent chapters should explain why. The EHC also recommends that concentrations be expressed in one constant unit throughout the document as well as in Table 1.

Every document must have a cut-off in time for completion. However, the International

1   Agency for Research on Cancer (IARC, 1998) has recently re-evaluated the evidence on the

2   carcinogenicity of 1,3-butadiene, and Health Canada is completing a health evaluation on the

3   compound. It will be important to include the IARC evaluation in an updated version of the health

4   assessment, and should the Health Canada document be finalized soon, it should also be included.

5   For example, Table 1-1 should include the IARC evaluation, and if available, the finalized Health

6   Canada evaluation (IARC, 1998). Also, the Agency should clarify, in the Table, whether the

7   OSHA classification listed 1,3-butadiene as a "known" or "potential" carcinogen.

8

9   It is stated that the profile for setting the American Conference of Governmental Industrial

10 Hygienists-Threshold Limit Value (ACGIH-TLV) for 1,3-butadiene is not reviewed because it is

11 not a risk assessment. This may be true for the EPA definition of a risk assessment, but the

12 Agency should include some history of what has been used for the work environment, particularly

13 because of the high values in the past and the importance of epidemiological studies on worker

14 population.

15

16   There should be a comment in the final document on the accessibility of unpublished data

17 that is referenced in Agency documents. This concern was based on the use of the

18 Delzell/University of Alabama at Birmingham (UAB) study in the Agency's health risk assessment

19 of 1,3-butadiene even though the review document was not readily available to the public

20 (Delzell, 1995).

21

22   Finally, there is no mention in the Introduction of EPA's proposed Cancer Risk

23 Assessment Guidelines. While these guidelines have not been completely finalized, they are

24 sufficiently complete to be referenced and utilized as appropriate (USEPA, 1996).

25

26

1   **3.1.2 Chapter 2 - Overview of Exposure to 1,3-Butadiene**

2

3   This chapter is intended to be an introductory review of possible sources of exposure to

4   1,3 butadiene. It should be clearly indicated in the first paragraph that this chapter is not intended

5   to be a comprehensive review of exposure so that this chapter is not mistakenly used as a source

6   of data for enforcement purposes.

7

8   An explanation should be included on how concentrations have been measured over the years and

9   how this might impact a comparison of exposure levels measured in earlier years with more recent

10   measurements. Ranges of concentrations are important to include along with averages. The

11   Committee recommends that all of the exposure concentrations be expressed in one common unit

12   throughout Chapter 2.

13

14   Additional points that need to be incorporated include:

15   1.

16   a)   *Section 2.2.1.* Butadiene monomer production facilities need to be discussed

17   especially since the epidemiology section discusses exposure in these facilities

18

19   2.   b)   *Section 2.2.1.* The impact of compliance with the 1994 Hazardous National

20   Emissions Standard for Hazardous Air Pollutants and the polymers and resins

21   Maximum Achievable Control Technology (MACT) on air emissions should be

22   discussed.

23

24   3.   c)   *Section 2.3.3.* This section discusses open burning of tires. Data on controlled

25   burning of tires should also be included and distinguished from open burning.

26   Specifically, results from a pilot study conducted by Paul Lemieux (1994),   US

27   EPA Air and Energy Engineering Research Laboratory should be   included. In

1   this study, 1,3 butadiene could not be detected from controlled combustion of

2   tire-derived materials.

3

4   4.   d)   *Section 2.4*. For a better perspective, where possible, tables should indicate

5   the number of samples that were below the detection limit as well as the

6   ranges of exposure concentrations. More recent data on emissions, such as the

7   1995 and 1996 Toxics Release Inventory (TRI), and soon to be released

8   EPA's 1996 nationwide emissions inventory for butadiene should be included

9   (USEPA, 1997, 1998b). If available, exposure concentrations **inside**

10   automobiles should be added.

11

12   e)   *Section 2.5*. In this discussion of exposures, similarities and differences of general

13   population and occupational cohort exposures should be discussed so   that the

14   limitations of extrapolation from occupational setting to exposure of   the general

15   population is understood. For example, short peak exposures in industrial setting

16   vs. chronic low levels of exposure to general population may impact extrapolation

17   of occupational data.

18

19   **3.1.3 Chapter 3 - Metabolism and Pharmacokinetics**

20

21   Chapter 3 reviews the metabolism and pharmacokinetics of 1,3-butadiene. This chapter is

22   long, contains numerous incorrect or incomplete statements that need to be changed, and should

23   be updated. The title of the chapter should be *Metabolism and Toxicokinetics*, as butadiene is not

24   used as a therapeutic agent. Also, the word, toxicokinetics, should replace pharmacokinetics

25   throughout the document.

26

27

1　The Health Canada assessment of butadiene and Dr. Himmelstein's submitted comments

2　will provide many of the new references that need to be incorporated into the revision. Specific

3　issues that should be addressed in the revision of the risk assessment are provided below (Health

4　Canada, 1997) (Himmelstein, 1998).

5

6　In evaluating the data on the metabolism of butadiene and its epoxide metabolites across

7　species, including humans, the EPA concluded that there are no clear species differences in

8　butadiene metabolism and that in many cases there is overlap in metabolic rates across species. In

9　forming their conclusion, the EPA misinterpreted the in vitro data and ignored the major

10　conclusion from the in vivo data. With respect to the in vitro data, the EPA compared maximal

11　rates of metabolism (i.e., V_{max}) rather than basing their comparisons on a more appropriate

12　measure of metabolic rates, the ratio of V_{max}/K_m. This ratio is more appropriate because a) species

13　differences are observed in both metabolic parameters , and b) at non-metabolically saturating

14　concentrations (i.e., those concentrations relevant for humans) it is the ratio, not the maximum

15　rate, that dictates the rate of metabolism. With respect to the in vivo data, the EPA has not

16　adequately summarized the data from two independent laboratories that clearly and

17　unambiguously show large differences spanning orders of magnitude in the blood and tissue

18　concentrations of the butadiene metabolite diepoxybutane. In particular, Table 3-8 is misleading.

19　This table includes data from the rat, mouse, and monkey for blood epoxybutene and

20　diepoxybutane concentrations from studies conducted by Bond et al. (1986) and Dahl et al.

21　(1991) which did not use gas chromatography-mass spectrometry (GC-MS) methods to

22　quantitate these metabolites (Bond, et al., 1986)(Dahl, et al., 1991). Therefore, the values

23　reported by these investigators are unreliable. It would be preferable to, instead, compare the

24　blood concentrations reported by Himmelstein, et al., Bechtold et al., and Thorton-Manning et al.

25　in one table (Himmelstein, et al., 1994, 1995))(Bechtold, et al., 1995)(Thorton-Manning et al.,

26　1995a, 1995b, 1996). This comparison would serve two purposes. First, it would highlight the

27　interlaboratory reproducibility in the values reported for these metabolites serving to increase the

11

1   reliability of the data. Second, it would accentuate the dramatic species differences in the

2   circulating levels of diepoxybutane, lending support to the hypothesis that this interspecies

3   difference in metabolite formation underlies the differences in susceptibility observed in the

4   chronic studies.

5

6   Mutagenicity studies strongly suggest that diepoxybutane is **a**, if not, **the** critical

7   metabolite in butadiene carcinogenicity. Thus, differences in levels of this metabolite formed in

8   vitro and in vivo are highly consistent with the observed species differences in carcinogenicity.

9   The Committee recommends that the Agency integrate and assess this information, develop

10   conclusions based on the weight of all the information presented, and present these conclusions in

11   the closing paragraphs of Chapter 3.

12

13   It is recognized that the EPA elected a cutoff date for inclusion of new information of

14   January 31, 1997. However, there is no question that a significant amount of new and important

15   information has been developed since this date that is particularly pertinent to Chapter 3 on

16   Metabolism and Pharmacokinetics. In short, the chapter on Metabolism and Pharmacokinetics

17   needs to be updated to include key references that have been published in the peer-reviewed

18   literature since January 31, 1997. Many of the data sets that have been published since this date

19   lend further support to the hypothesis that diepoxybutane is a critical metabolite involved in the

20   carcinogenicity of 1,3-butadiene.

21

22   While this chapter references important literature relative to the in vitro and in vivo

23   metabolism and butadiene, it falls short in providing a comprehensive integration of the relatively

24   diverse data sets. A critical concept that appears lacking in this chapter is the fact that

25   diepoxybutane represents a critical metabolite of butadiene and that there are significant species

26   differences both in vitro and in vivo in the formation of this important butadiene metabolite. This

27   chapter neglects to note that metabolism and toxicokinetic studies of butadiene conducted in

1   whole animals and in rodent and human tissues provide important insights into the likely critical

2   steps in the initiation of butadiene carcinogenicity and importantly the identity of the most likely

3   chemical species responsible for the development of tumors. For example, dosimetry data on both

4   epoxybutene and diepoxybutane following inhalation exposure to butadiene clearly indicate that

5   blood concentration of epoxybutene were up to 8-fold higher in mice compared with rats and that

6   blood concentrations of diepoxybutane were nearly 40-fold higher in mice than in rats. Similarly,

7   tissue concentrations of epoxybutene ranged from 3-10 times higher in mice compared with rats

8   and tissue concentrations of diepoxybutane were up to 100 times higher in mice than rats.

9   Importantly, therefore the correlation between measured circulating blood and tissue levels of the

10   epoxides, particularly diepoxybutane and the observed development of tumors is clearly

11   suggestive of a role of diepoxybutane in the initiation of cancer. This important concept is not

12   presented in the chapter.

13

14   Moreover, in vitro data on the metabolism of butadiene suggest that mice form

15   epoxybutene and diepoxybutane at a faster rate than rats or humans. Studies on the in vivo

16   metabolism and tissue concentrations of epoxybutene and diepoxybutane in mice and rats

17   following inhalation exposure to butadiene are consistent with the in vitro studies on metabolism

18   of butadiene. This is an important point and the chapter neglects to point out the close parallels

19   between the observations in in vitro studies and in vivo studies.

20

21   The Agency notes that butadiene is an animal carcinogen and that the mouse is more

22   sensitive than the rat to butadiene induced carcinogenicity. The Agency also notes that the reasons

23   for these interspecies differences are not understood at this time. However, the available

24   mechanistic data on the metabolism of butadiene and its reactive epoxide metabolites supports the

25   hypothesis that interspecies differences in metabolic rates form the underpinning for the increased

26   sensitivity of the mouse compared with the rat. These differences in metabolic rates result in a

27   faster production and slower detoxification of the diepoxybutane in mice compared with rats with

1   resultant higher steady state levels of the diepoxybutane in blood and tissues in the mouse

2   compared with the rat following exposure to butadiene. This observation coupled with the

3   demonstrated hundred-fold greater mutagenicity of the diepoxybutane compared with the

4   epoxybutene points to interspecies differences in the formation of diepoxybutane being critical to

5   interpretation of differences in response.

6

7   The Agency notes on page 11-9 of the document that the specific mechanisms of

8   1,3-butadiene induced carcinogenesis are unknown. As noted above, there is extensive

9   mechanistic data on the metabolism of butadiene in various species, including humans, that

10   suggest that the extreme sensitivity of the mouse is related to the excessive formation of the

11   diepoxide metabolite in this species. Experimental studies show that both humans and rats

12   produce much less of the metabolite.

13

14   Additional points that should be incorporated include:

15

16   a)   *Page 3-1, Line 15*: Much of the literature used the abbreviation BD for 1,3-

17   butadiene. It should be less confusing if BDiol is used for the 3-butene-1,2-diol.

18   This section does not even mention the 3,4-epoxy-1,2-butanediol (EBD). The

19   latest research on molecular dosimetry strongly suggests that EBD is the major

20   electrophile that binds to DNA and hemoglobin. EBD should be readdressed in the

21   final version of this risk assessment.

22

23   b)   *Page 3-2, Line 14*: Recent data shows that the trihydroxybutane (THB) adducts

24   are clearly the predominate adducts in DNA and hemoglobin. These can arise from

25   either 3,4-epoxy-1,2-butanediol or 1,2:3,4-diepoxybutane (DEB). Based on tissue

26   measurements of DEB and EB, the ratios of epoxybutene (EB) to

27   DEB/EBD(THB) adducts strongly suggest that EBD is the primary source of the

1   THB adducts. The EBD metabolite has not been quantitated following *in vivo*

2   exposure. This represents a major gap in our knowledge that needs to be

3   acknowledged.

4

5   c)   *Page 3-2, Line 14-18*: The toxicokinetic data clearly does not support the

6   statement that the possible crotonaldehyde metabolites are "Of greater

7   significance." This editorial comment should be removed from the document, since

8   no causal role of these metabolites in butadiene mutagenicity or carcinogenicity has

9   been shown.

10

11   d)   *Page 3-3*: This chart has several errors that need correction. 3-Butene-1,2-diol is

12   not reactive and should not have a box around it. Furthermore, the arrow for

13   Reaction 11 is going in the wrong direction. Crotonaldehyde and acrolien are

14   reactive and should have boxes around them. The urinary metabolites M-I and

15   M-II should be shown.

16

17   e)   *Page 3-27*: (9) This reaction seems to be mislabeled. It appears that this refers to

18   BDiol GSH-BDiol.

19

20   f)   *Page 3-38, Line 21-22*: Most 1,3-dihydroxypropanone probably comes from EBD.

21   This statement gives a wrong impression.

22

23   g)   *Page 3-39, Line 18-25*: The toxicokinetics of butadiene are much more

24   complicated than discussed. The molecular dosimetry of DNA adducts following 4

25   weeks of exposure clearly shows that the first oxidation to EB and its subsequent

26   binding to DNA is linear over a range of 20-625 ppm in rats and mice (Swenberg,

27   et al., 1998). It is rapidly converted to BDiol and then to EBD. To a lesser extent,

1   EB is oxidized to DEB. Both EBD and DEB form THB adducts, although most of

2   these come from EBD. The formation of THB adducts is saturated at 62.5 ppm in

3   the rat, so that exposure to higher amounts such as 1000-8000 ppm results in little

4   more THB adducts than does exposure to 62.5 ppm. In contrast, the mouse shows

5   a biphasic response for the formation of THB adducts, with a steep slope between

6   0 and 62.5 ppm and a lesser slope from 62.5-625 ppm. The mouse does not show

7   total saturation of the formation of THB adducts, suggesting that it has a second

8   enzymatic pathway that is still active at high exposures. These data were presented

9   at the SAB meeting, were presented at the Society of Toxicology (SOT) and

10   Health Effects Institutes (HEI) annual meetings in 1998, and will be submitted for

11   publication this summer (Tretyakova, et al., 1998)(Swenberg, et al., 1998).

12

13   h)   *Page 3-40, Line 13-14*: This sentence is unclear. What is metabolic capacity of

14   EB? Is this formation or further metabolism? Available DNA data suggests that

15   formation continues to occur in an exposure related manner.

16

17   I)   *Page 3-41, Line 8*: Butene diol is not a hydrolysis product of <u>DEB</u>. This should be

18   EB.

19

20   j)   *Page 3, Line 41-42*: The sections on DNA and hemoglobin adducts are seriously

21   out of date, as this is a fast moving area of research. Data from several laboratories

22   have shown that the THB adducts are predominant over EB adducts. At high

23   concentrations of butadiene (625-1000 ppm), the ratio of THB/EB is 1.5-4 for rats

24   and 3-9 for mice. This drastically changes at nonsaturating exposures of 20 ppm

25   and 62.5 ppm, where rats have 27.5 and mice have 43-47 times more THB

26   adducts. The same finding has been demonstrated for human hemoglobin adducts.

27   THB-Valine adducts are formed about 40 times more frequently than EB-valine

1   adducts. The EPA document only mentions EB adducts. A molecular

2   epidemiology study of hemoglobin adducts was recently completed at the National

3   Cancer Institute (NCI) in a Chinese butadiene worker study coordinated by Dr.

4   Richard Hayes. Nearly all blood samples examined, whether from exposed or

5   unexposed individuals, had measurable THB-Valine adducts. The number of

6   adducts in unexposed individuals averaged ~40 pmol/g globin. There are similar

7   data for U.S. research workers. With exposure to butadiene estimated to be 1-3.5

8   ppm, the number of adducts increased 2-3-fold ($R^2 = 0.33$). Of interest is the

9   finding that glutathione S-transferase theta (GSTT1) genotype had no effect on

10   THB-Valine adducts. Since the GSTT1 null genotype has clearly been associated

11   with increased susceptibility to DEB-induced sister chromatid exchanges (SCEs)

12   and is primarily located in erythrocytes, this observation supports EBD as the

13   primary human electrophile forming THB-Valine adducts.

14

15   k)   *Page 3-45*: The section on Discussion and Conclusions should be revised. Lines

16   8-10 are oversimplified. Line 12 is wrong. The 1,3-butadiene epoxide should be

17   butene diol. Lines 19-23 clearly do not reflect molecular dosimetry data and

18   actually appear to be reversed. Line 27 is wrong. Butene diol is not toxic. EBD

19   needs to be added to this section, as it is likely to be a major metabolite.

20

21   This section of the risk assessment should also address what is known about genetic

22   polymorphisms that are likely to affect individual susceptibility to butadiene and its metabolites.

23   Several genes appear to be important. Inherent susceptibilities have been shown for both DEB and

24   EB (Weincke and Kelsey, 1993), which may be due to glutathione S-transferase theta (GSTT1)

25   status. Also, glutathione S-transferase μ(GSTM1) appears to be an important detoxifying factor

26   for EB, so that GSTM1 null individuals would be expected to have greater effects following

27   formation of EB. Unfortunately, no data have been published on the effects of GST

1   polymorphisms on EBD. This is a gap in our knowledge. Genetic polymorphisms have also been

2   identified for epoxide hydrolase (EH) and CYP 2E1 that would be expected to affect

3   susceptibility to butadiene and its metabolites. The role of these proteins in the toxicokinetics of

4   numerous chemicals is reasonably well known. Three studies (Csanday, et al., 1992; Seaton et al.,

5   1995, and Duescher and Elfarra, 1994) have shown in vitro using rodent and human tissue

6   samples that CYP2E1 plays a role in the oxidation of both BD and EB. It is possible to expect

7   that polymorphisms that reduce EH activity will increase susceptibility to butadiene. Likewise,

8   rapid CYP 2E1 metabolizers would be expected to be at greater risk.

9

10

1   **3.1.4 Chapter 4 - Mutagenicity**

2

3   This chapter summarizes the genotoxicity of 1,3 butadiene (BD) and its metabolites for

4   mice, rats and humans, considering both cytogenetic endpoints and gene mutations in somatic and

5   germinal cells. The emphasis is on recent in vivo studies, although there is some mention of results

6   from in vitro assays. Most of the extensive work prior to 1994 is not mentioned. However,

7   citations are given for the numerous relevant reviews. The recommendations for Chapter 4 are

8   given below.

9   a)   Text tables summarizing key animal and human findings derived from the entire

10   body of information on BD should be added.

11

12   b)   References to support key findings should be included in the tables.

13

14   c)   Separate tables should be included for in vitro, animal and human findings.

15

16   d)   Similarities and species differences in response should be noted.

17

18   e)   More emphasis should be given to the positive heritable translocation studies in

19   mice because of their potential relevance for human heritable risks, and to several

20   additional studies conducted in humans. This recommendation is also discussed in

21   Section 3.1.5 which addresses the *Reproductive and Developmental Effects*

22   chapter of the review document.

23

24   f)   Some of the key findings that should be added or expanded are discussed in detail

25   below. The conclusion section should be expanded to include what is known about

26   the mutagenicity of BD and its metabolites from the extensive literature. Careful

27   editing of the final document should be conducted to avoid missing dose units,

1   units for mutant frequencies, and similar omissions.

2

3   g)   *BD Metabolites Bind to DNA*

4   i)   Electrophilicity is an important property of chemical mutagens. The

5   evidence that BD's metabolites react with proteins such as hemoglobin is presented

6   in Chapter 3 of this draft document. Evidence for reactivity   with the DNA

7   itself demonstrates that these BD intermediates reach their   target molecule for

8   genotoxicity causing premutagenic DNA lesions.   Some of this evidence is

9   also reviewed in Chapter 3, but should be   expanded greatly in this chapter

10   on mutagenicity.

11

12   ii)   Early studies showed that DEB binds to N-7 guanine and that it forms inter-strand

13   crosslinks (Brooks and Lawley, 1961; Lawley and Brooks,   1967).

14   Subsequently it was shown that B6C3F1 mice and Wistar rats   exposed to [14]C

15   labeled BD by inhalation have covalently bound reactivity   in liver DNA, as

16   noted in Chapter 3 (Kreiling et al., 1986). The amounts   bound in the two

17   species were comparable.

18

19   iii) The complex kinds of adducts formed in DNA by BD metabolites are also

20   under active investigation. Specific enantio- and regioisomeric EB   adducts

21   formations have been shown (Koivisto et al., 1997; Tretyakova et   al., 1997).

22   The N-7 position of guanine has been shown to be the most   reactive with EB,

23   followed by the N-3 and N-1 positions of adenine. EB   adducts have also been

24   found at the N-6 position of adenine but this may   represent a rearrangement of

25   the N-1 adenine adduct. Adenine adducts   may be important for the

26   genotoxicity of BD as shown by mutational   spectral studies of this agent.

27

1

2   iv)   The several nucleobase adducts in DNA formed by BD metabolites are

3   being characterized further by several groups (Leuratti et al., 1994; Neagu

4   et al., 1994, 1995; Selzer and Elfarra, 1996; Kumar et al., 1996).

5   Furthermore, attempts are being made to use high sensitivity methods for

6   detecting DNA adducts in humans for biomonitoring purposes, including

7   the detection of urinary DNA adducts. However, despite the statement

8   made in Chapter 11, page 11-9, para. 1, line 2, the Committee is not

9   aware that DNA adducts have even been observed in vivo in humans. The

10   statement in this regard made on page 11-9 either needs a reference or

11   should be corrected.

12

13   h)   *Extensive Genotoxicity Profile for BD and its Metabolites*

14   The extensive mutagenicity results covered in the several reviews cited in Chapter

15   4 include positive Salmonella assays in the presence of S9. It is important to note

16   that human S9 also converts BD to mutagenic metabolites, as determined in the

17   Salmonella system (Arce et al., 1990). BD metabolites also have been positive   for

18   mutagenicity in a variety of microorganisms with or without metabolic activation.

19   Studies of mutation by BD at the tk locus in mouse lymphoma cells have been both

20   positive (Sernau et al., 1986) and negative (McGregor et al., 1991). BD, as

21   opposed to its metabolites, has not been found mutagenic in vivo in Drosophila

22   melanogaster, as assessed by the sex-linked recessive lethal mutation assay

23   (Foureman et al., 1994) or by the Wing Spot Test (Victorin et al., 1990).

24

25   i)   *Comparative Genotoxicity Results in Mice and Rats*

26   There have been numerous cytogenetic studies in vivo in rodents. These are

27   covered in the cited reviews and, as correctly stated in Chapter 4, support the

21

1   dichotomy in carcinogenic response between these two species, where mice are

2   more responsive than rats. However, it should be noted that, with regard to

3   cytogenetic assays in vivo in these two species, there are no reports of positive

4   results in rats exposed to BD (as exposed to BD metabolites) but there are several

5   reports of positive results in mice.

6

7   j)   *Contrast in clastogenicity results between species*

8   i)   The difference in BD's apparent mutagenic potencies for mice and rats are

9   worthy of comparison. Studies can be considered as those measuring

10   clastogenicity and those measuring specific gene mutations. In regards to

11   clastogenicity, for mice there are numerous reports of chromosome

12   aberrations, micronuclei and SCEs in somatic cells, i.e. in both lymphocytes

13   and in bone marrow cells, several showing effects at low doses. However,

14   in rats, neither chromosome aberrations nor micronuclei have been found in

15   blood cells in vivo (i.e. in lymphocytes, bone marrow or peripheral red

16   blood cells) after BD inhalation (Arce et al., 1990; Autio et al., 1994),

17   although there is a report of a weak positive SCE response

18   (Maki-Paakkanen et al., 1993).

19

20   ii)   For germ cells, the contrast in clastogenicity results between these species

21   is equally striking. In mice, there are reports of dominant lethal effects with

22   inconsistent results (references given in the draft document), sperm head

23   abnormalities (Morrissey et al., 1990), micronuclei in spermatids (Xiao and

24   Tates, 1995; Tommasi et al., 1998) and a positive Comet assay in haploid

25   and polyploid testicular cells (Brinkworth et al., 1998). The dominant lethal

26   studies discussed in Chapter 5 should be cross-referenced in Chapter 4 as

27   they are important to the discussion of heritable chromosomal alterations

1   induced by BD. Cytogenetic abnormalities in first-cleavage embryos sired

2   by male mice treated by BD inhalation have also been reported recently

3   (Pacchierotti et al., 1998a). By contrast, in the single study in rats, no

4   dominant lethal mutations were found (BIBRA, 1996).

5

6   iii)   Of special importance among the studies of clastogenicity are those of

7   heritable translocations in mice. The first study is discussed briefly in

8   Chapter 4 (Adler et al., 1995). A second study by the same group has

9   recently been reported (Adler et al., 1998). These studies are particularly

10   relevant to human health and risk assessments for human heritable damage

11   and should be emphasized. In fact, a human risk estimate has recently been

12   reported using a parallelogram approach that employed mouse somatic cell

13   clastogenicity (micronuclei in bone marrow cells), mouse germ cell

14   clastogenicity (the heritable translocations) and human somatic cell

15   clastogenicity (in lymphocytes from BD exposed workers) to estimate the

16   heritable translocation risk for humans (Pacchierotti et al., 1998b). The

17   estimated doubling dose for human heritable translocations was given as

18   1,100 ppmh (parts per million hours)

19

20   k)   Comparison of the specific locus mutagenicity of BD between mice and rats is not

21   nearly as striking as is the comparison for clastogenicity. The studies summarized

22   by Recio and Goldsworthy (1995) showing an increase in BD-induced lacI

23   mutations at A:T base pairs in bone marrow stem cells from young transgenic

24   B6C3F1 (BB) male mice are discussed in Chapter 4. However, it should be

25   mentioned that an earlier study in young male Balb/c X DBA/2 (CD2F1)

26   transgenic mice (MM) found lacZ BD induced mutations only in lung cells and not

27   in liver or bone marrow stem cells (Recio et al., 1992). There is also a report of a

1   positive spot test in "T" stock mice that found an increase in in vivo mutations in

2   embryonic melanoblasts (Adler et al., 1994).

3

4   l)   There are several reports of positive hprt mutations in young B3C3F1 mice

5   exposed to BD by inhalation. The Cochrane and Skopek (1994b) study

6   mentioned in Chapter 4 exposed pre-weanling mice and found dose-related

7   mutational increases. Again, there was a bias for A:T changes. The Meng study

8   mentioned in Chapter 4 (Meng, et.al, 1996) also exposed young B6C3F1 male

9   mice to BD and found increases in hprt mutations in both thymic and splenic

10   lymphocytes that persisted for several weeks after the exposures. This work has

11   now been accepted as a full publication and indicates that the hprt mutant

12   frequency (MF) in thymic lymphocytes rises from 2.2×10^{-6} to 11.3×10^{-6} at two

13   weeks (maximal) and, in splenic lymphocytes, from $\sim 1.8 \times 10^{-6}$ to 19.7×10^{-6} at

14   five weeks (maximal). The mutagenic potency of BD in these mice, which is a

15   measure of hprt mutant cell accumulation, was calculated to be 69.62.

16

17   m)   There are also negative reports for in vivo hprt mutations in other strains of mice

18   that were older at the time of their BD exposures. Tates et al. originally found low

19   order hprt mutagenicity in splenic lymphocytes of 10-12-week-old (102/E1 X

20   C3H/E1) F1 mice (Tates et al., 1994), but later failed to find an increase in such

21   hprt mutations in a second study of this strain or in adult CD1 mice (Tates et al.,

22   1998).

23

24   n)   In contrast to the clastogenicity studies, specific locus gene mutations have been

25   found in vivo in rats exposed to BD by inhalation. Meng et al., 1996 exposed

26   young rats with results as noted in Chapter 4, i.e. increases in hprt mutations in

27   thymic lymphocytes from 2×10^{-6} to 4.9×10^{-6} at three weeks after exposure

24

1   (maximal) and, in splenic T-cells, from ~1.9 x 10^{-6} to 10.1 x 10^{-6} at four weeks

2   after exposure (maximal) (references given in chapter). The mutagenic potency of

3   BD in rats was calculated to be 15.85. Thus, the ratio of mutagenic potencies

4   between mice and rats (mice/rats) was calculated to be 4.4 or 5.0, depending on

5   the weeks allowed for mutant cell accumulation. Thus, although BD is 5X more

6   mutagenic in rats, the magnitude of this difference is not nearly as great as that

7   reported for the carcinogenicity differential between these two species.

8

9   o)   *Studies of BD Metabolites*

10   The many rodent studies of BD metabolites, some positive and some negative,

11   show a similar pattern. In general, younger animals tend to give the positive results

12   and, for mice, it is the B6C3F1 animals that are most often associated with positive

13   studies. The Cochrane and Skopek studies of EB and DEB were noted in Chapter

14   4. Meng et al. have extended their studies in young mice (B6C3F1) and rats

15   (Fischer) to EB and DEB by inhalation and have shown increased hprt mutations

16   in splenic TÄcells in both species (Meng et al., 1997). In rats, however, the

17   response to EB was equivocal. In contrast to these positive results, Tates et al.

18   found no hprt mutation induction in older mice (102/E1 X C3H/E1) F1 and CD1

19   or rats (Lewis) administered EB or DEB by injection or in the drinking water.

20   However, as noted in Chapter 4, rat germinal cells are at least as susceptible (or

21   more so) to the clastogenicity of BD metabolites as are mouse germinal cells.

22

23   p)   *Summary of In Vivo Rodent Studies*

24   i)   In summary, there are striking species differences between mice and rats in

25   the reported studies as regards clastogenicity. There are numerous positive

26   studies of this endpoint in mice, but BD induced clastogenicity has not been

27   demonstrated in vivo in rats. Of note, heritable translocations have been

1   demonstrated and confirmed following BD exposures to mice.

2   Clastogenicity has been observed in mice and rats exposed to EB or DEB.

3

4   ii)   For gene mutations, B6C3F1 mice appear to be more sensitive than other   mouse

5   strains to the mutagenic effects of BD, EB or DEB. In general,   mice are

6   also more sensitive than rats to specific gene mutations, although   such

7   mutations can be induced in younger rats. Mutations are most   commonly

8   seen in both species in young animals, indicating that cell   proliferation may be

9   required to produce these mutagenic effects.

10

11   q)   *Human Studies*

12   i)   Chapter 4 includes most of the relevant mutagenicity (clastogenicity and

13   specific gene mutations) studies in human cells and/or in vivo in humans.

14

15   The Cochrane and Skopek study (1994) of hprt and tk mutations in vitro in

16   human TK6 cells described in page 4-1 should note that mutation induction

17   was assessed for EB, DEB and for EBD . The mutagenic potencies of

18   these BD metabolites were found to be DEB> EB>EBD. It is important to

19   note that the metabolite EB diol has been studied and has been found to be

20   mutagenic. The importance of EBdiol lies in its abundance. Therefore, even

21   though Ebdiol(EBD) may be the least mutagenic of the metabolites, it may

22   be the most abundant, and therefore may give the most mutations.

23

24   ii)   Some of the in vivo cytogenetic studies in humans are covered in Chapter

25   4. The positive challenge assay reported by Au et al., 1995 is noted as is

26   the negative report of chromosome aberrations, micronuclei and SCEs in

27   exposed workers by Sorsa. Not noted, however, is the update by Sorsa

1　　　　　　　(Sorsa et al., 1996) that reanalyzed these data according to GST T1 status

2　　　　　　　and found that the T1 null workers appeared to have increases in chromosome

3　　　　　　　aberrations. Also, the increases in chromosome aberrations　　but not in

4　　　　　　　frequency of micronuclei reported by Tates in 1996 (and noted　　　in Chapter

5　　　　　　　4) has been updated in a full manuscript that reports increases　　　in both

6　　　　　　　chromosome aberrations and SCEs in BD exposed Czech workers. The

7　　　　　　　frequencies of micronuclei were not increased and the Comet assay　　was

8　　　　　　　negative (Tates et al., 1998).

9

10　　iii)　　　The in vitro findings of increased EB induced SCEs in lymphocytes from

11　　　　　　　GST M null individuals and increased DEB induced SCEs in lymphocytes

12　　　　　　　from GST T null individuals are reported in Chapter 4 and are important,

13　　　　　　　as is the observation that GST status does not appear to affect SCE

14　　　　　　　frequencies induced by EBD. A recent in vitro study observed chromosome

15　　　　　　　specific aneuploidy (for chromosomes 12 and X) in human lymphocytes in

16　　　　　　　vitro following treatments with either EB or DEB (Xi et al., 1997). The

17　　　　　　　paper by Wiencke et al, (1995) demonstrating the affinity of the glutathione

18　　　　　　　transferase theta enzyme for the diepoxide metabolite of BD should be

19　　　　　　　added.

20

21　　iv)　　　The status of the in vivo hprt mutation studies in humans has been

22　　　　　　　accurately reported in Chapter 4. There are the three positive studies using

23　　　　　　　the autoradiographic assay, as noted, and the two negative studies that

24　　　　　　　employed the cloning assay. Chapter 4 discusses the discordance in these

25　　　　　　　two groups of studies and concludes that, regardless of the reason for the

26　　　　　　　difference, the positive results with the autoradiographic assay probably

27　　　　　　　reflect a mutagenic effect of BD in the monitored workers. . There was a

1   difference in opinion among the Committee regarding the hprt results.

2   Some of the Committee found the Agency's conclusions to be reasonable

3   while other Members/Consultants were concerned that the

4   autoradiographic results may be method-related.

5

6   v)   The explanation as to possible reasons for the discordance between the

7   positive effects obtained by autoradiography and the negative results found

8   by cloning for human BD induced hprt mutations in vivo however should

9   be rewritten. In considering the differences between the Ward et al., 1994,

10   1996 positive autoradiographic studies and the Hayes et al., 1996 and

11   Tates et al., 1996 negative cloning assay studies, the draft document

12   concludes that "a simple explanation would be that the increase in the

13   autoradiographic assay was due to clones of mutants having arisen from

14   earlier mutations." There was a diversity of opinion regarding the

15   discordance between the positive effects obtained by the autoradiography

16   and the negative results found by cloning for human butadiene inducted

17   hprt mutations in vivo. Some of the Committee found that Agency's

18   explanation is probably not correct for two reasons. First, the

19   autoradiographic assay requires a technical step before the T-cells can be

20   assessed for hprt mutations, i.e. cryopreservation or some measure to

21   arrest those few cell that are cycling in human peripheral blood at the G1-

22   S interphase. This is simply a technical means to insure that cycling non-

23   mutant T-cells do not progress to their S-phase in vitro in the presence of

24   6-thioguanine and become labeled - even slightly labeled - and thereby

25   become scored as mutants in the assay. Such cells are not mutants but

26   would appear to be so by virtue of their (partial?) labeling, i.e. they are

27   phenocopies. This phenomenon, which artifactually elevates scored variant

1   frequencies, can be eliminated by arresting the cycling cells at this phase of

2   the cell cycle, from which they rapidly proceed into S in vitro before the

3   label is added. Thus, they miss the scoring window and are not scored as

4   variant cells.

5

6   vi)   The reason why this arresting or cryopreservation step is relevant to the

7   issue of clonality is that T-cells that are undergoing clonal expansions in

8   vivo tend to be among the cycling cells. If such large mutant clones were

9   present, the cycling members of the clones would be eliminated from the

10   scoring window by the cryopreservation step (even though these would be

11   mutants). Therefore, if anything, clonal amplifications are less likely in the

12   autoradiographic than in the cloning assay. Said the other way around, if in

13   vivo clonality were the reason for the higher mutant frequencies in exposed

14   vs. control workers, this phenomenon would have most likely affected the

15   results in the cloning assay. This is the exact opposite to what was

16   observed, i.e. the increased mutant frequencies in exposed over controls

17   were observed with the autoradiographic assay.

18

19   vii)   Another reason why in vivo clonality cannot account for the differences in

20   results between the assays is that molecular studies were not done. Such

21   studies cannot be done for the autoradiographic assay but can when using

22   the cloning assay. Molecular analyses allows detection of in vivo   clonality

23   using the cloning assay. Since no such studies were done, no "corrections"

24   were made and the point is moot.

25

26   viii)   There are other differences between the autoradiographic and cloning

27   assays that could account for the differences in results obtained with these

1   two assays. The autoradiographic method is a phenotypic assay, meaning

2   that mutants (or variants) are consumed by the assay and, therefore, cannot

3   be analyzed at the molecular level. It is not, therefore, possible to

4   demonstrate that the observed mutants scored by this assay are actually

5   genotypic mutants. This, however, is not different from other phenotypic

6   assays for mutations that are in wide use. To be scored in the

7   autoradiographic assay, variant cells have simply to synthesize DNA in

8   their first S in vitro. No cell division or growth is required. In addition, the

9   cells must begin their DNA synthesis within 36-48 hours in culture.

10   Therefore, a quite different T-cell subpopulation may be scored in the

11   autoradiographic than in the cloning assay. It is possible that, in the former,

12   only that subset of cells that are capable of division quickly in vitro is

13   scored whereas, in the cloning assay, all T-cells are scored. Alternatively,

14   what is being scored in the autoradiographic assay may not be fixed

15   mutations but rather adduct-blocked RNA transcription. Another

16   possibility is that the cell that would go into a G2 block, and therefore not

17   be measured by the cloning assay, are scored in the autoradiographic assay

18   because they are not required to go through G2 for scoring.

19

20 ix) The autoradiographic assay requires that peripheral blood T-cells be

21   cryopreserved in order to arrest cycling cells at the G1 S interphase. If all

22   cycling cells are not eliminated, the autoradiographic assay might give an

23   artifactually elevated reading because of the scoring of phenocopies. This is

24   explained above.

25

26 x)   As for all mutation assays, the autoradiographic assay may have a scoring

27   bias. However, the cloning assay too has an inherent observer bias and a

1   tendency of some technicians to score only larger mutant colonies.

2

3   xi)   In addition, with respect to the BD studies, the differences between the

4   positive and negative studies may be due, in part, to differences in study

5   populations. The Ward et al. 1994, 1996 radiographic studies were

6   conducted at a butadiene monomer production facility and an SBR

7   production facility in Texas. The Tates et al., 1996 study was conducted in

8   the Czech Republic (butadiene monomer production) and the Hayes et al. ,

9   1996 study in China (polybutadiene rubber production). Smoking and other

10   lifestyle and confounding parameters would be different among these

11   populations. In addition, the exposure assessment in these studies were not

12   precise and no attempt was made to determine mutation susceptibility

13   profiles of the monitored workers. (It is of note, however, that one of the

14   highest variant frequency values in the Ward et al., 1996 study was in a

15   GST T null individual.)

16

17   r)   *Conclusions*

18   Few conclusions are made at the end of Chapter 4. The quality of the conclusions

19   would be improved by expanding this section and adding statements summarizing

20   what we know about the mutagencity of BD and its metabolites. The following list

21   could serve as a guide:

22

23   i) Rodent studies indicate that some of the metabolites of BD are DNA   binding

24   agents.

25

26   ii)   There are numerous in vitro assays in several model systems that

27   demonstrate the genotoxicity of BD and/or its metabolites.

1

2   iii)   In vivo studies in mice and rats show that the former is the more sensitive

3   species for the genotoxicity that follows exposure to the parent BD.

4

5   iv)   Young animals (including mice), and perhaps certain strains within a

6   species, are more susceptible to the genotoxicity of BD and/or its

7   metabolites than are others as indicated by hprt results.

8

9   v)   Clastogenicity has not been demonstrated for either somatic or germinal

10   cells in rats exposed to the parent BD.

11

12   vi)   Heritable translocations are induced by exposure of male mice to BD by

13   inhalation. (This is an important point for estimating heritable risks.)

14   Equivalent data are not available in the rat. Any estimation of human

15   heritable risk must account for species differences in metabolism.

16

17   vii)   BD metabolites give genotoxic effects in both rats and mice with mice

18   being the more sensitive to somatic effects but both species being equally

19   susceptible (or rats more so) to the germinal effects.

20

21   viii)   Human studies have shown that BD metabolites cause gene mutations in

22   vitro in human cells.

23

24   ix)   GST genotypes may affect susceptibility to the clastogenicity (and possibly

25   mutagenicity) of BD metabolites.

26

27   x)   There is evidence of clastogenicity in vivo in human lymphocytes from

1   exposed workers.

2

3   xi)   Hprt mutations have been shown in vivo in human T-lymphocytes of

4   exposed workers as documented by the autoradiographic but not by the

5   cloning assay for these events.

6

7   **3.1.5 Chapter 5 - Reproductive and Developmental Effects**

8   It was evident to the Committee that the EPA had a large amount of material to review

9 since that there were over 20 animal bioassays on this topic area. In addition, many new studies

10 have come in after the cut-off date for the assessment and, as noted below, this research will need

11 to be included in the report.

12

13   a)   *Organization*

14   Because of the volume and the complexity of the studies and reports for

15   both endpoints, additional summary tables are necessary in Chapter 5.   All

16   pertinent studies that are discussed in this chapter should be introduced in

17   a summary table at the start of each section for developmental and

18   reproductive toxicity. A good example is Table 5-13 which was prepared

19   for summarizing the structure-activity information.

20

21   b)   *Inclusion of negative data*

22   Chapter 5 needs to include both positive and negative studies in the

23   assessment. For example, text for Table 5-13 says that "since no

24   non-neoplastic lesions were seen, then they were not included in the

25   table." The report should include positive and negative data so the

26   reviewer can develop a comprehensive understanding of the data base

27   supporting the assessment.

1

2   c)   *Inclusion of new data*

3   The health assessment needs to include more current research. Every

4   effort should be taken to extend the time for data inclusion to as close as

5   possible to the release date of the assessment. For example, because of the

6   cut-off date, several new dominant lethal studies were not included. These

7   2 new negative studies need to be included in the reassessment. The

8   BIBRA Study III (Brinkworth, M., et al., 1998), which was a repeat of

9   BIBRA I (BIBRA International, 1995), was negative and needs to be

10   considered in this health assessment especially since the BIBRA I study

11   was used in one of the quantitative assessments (BIBRA III) (BIBRA

12   International, 1996)(Brinkworth, M., et al, 1998). Written testimony from

13   M. Christian identifying and criticizing these studies should be considered

14   in the report (Christian, M., 1998).

15

16   d)   *Integration of Chapter 5 with other chapters*

17   i)   It was difficult for reviewers of this health assessment to integrate

18   the toxicological findings. A much greater emphasis on integrating

19   findings across the chapters is needed. For example, information

20   on toxicokinetics and metabolism needs to be fully integrated into

21   this chapter. The information on toxicokinetics and metabolism in

22   the review document appear to be almost an after thought.

23   Interesting data on specific ovotoxic metabolites are available, and

24   should be incorporated. The potential impact of including the

25   PBPK model in the assessment of reproductive and developmental

26   toxicity needs to be discussed. Does this help to explain dose

27   response differences across species for ovotoxicity? The Agency

1   should explain this in the assessment. It is important not only to

2   show data but to discuss the implication of the data.

3

4   ii)   Another example where integration is needed is with the general

5   toxicity chapter with Chapter 5. Was there any evidence for

6   neurotoxicity from general toxicity testing to suggest the need for

7   developmental neurotoxicity assessment? What about effects in

8   utero on ovarian or testicular development? The heritable

9   translocation data from Chapter 3 should be integrated with

10   Chapter 5.

11

12   iii)   Because of the potential interrelatedness of the reproductive tumors

13   and reproductive organ atrophy data, the reproductive chapter

14   should have some overlap with the chapter discussing tumor results

15   for these reproductive organs. Also, Chapter 5 should include a

16   discussion on the mechanisms responsible for atrophy and

17   reproductive tumors.

18

19   e)   *Biological significance of atrophy/dominant lethals*

20   This chapter should provide discussion on the biological significance of

21   ovarian and testicular atrophy. This is especially true as these endpoints

22   are modeled in Chapter 9. How are exposed rodents different than control

23   rodents? The Agency should explain its rationale for looking at ovarian

24   atrophy in aged rodents.

25

26   f)   *Additional information*

27   The following issues should also be addressed in Chapter 5.

1   i)   The reader needs to understand the rationale for modeling the

2   dominant lethal data. The Agency should provide discussion that

3   supports the use of these endpoints in Chapter 9. Since new studies

4   on heritable translocations are available, these findings should be

5   integrated with the chapter on mutagenicity. This will provide

6   stronger evidence for the potential of this compound or its

7   metabolites to produce heritable and multi-generational impact.

8   The EPA should integrate the data so that the total picture of

9   research supports your identification of critical endpoints and

10   quantitative modeling.

11

12   g)   *Uncertainty*

13   For both the reproductive and developmental endpoints, it is necessary to

14   list all assumptions, identify where the assessor is uncertain and identify

15   agency action taken to respond to that uncertainty. The Agency should

16   explain what the critical data needs are that will address this uncertainty

17   and should be as specific and explicit as possible.

18

19   h)   *Use of speculative and editorial type of sentences*

20   The Agency should avoid the use of speculative and editorial types of

21   sentences. A specific example is on Page 5-28, lines 32-33, the assessment

22   speculates that the compound does not cross the blood-testis barrier. Why

23   does the Agency think that this is true? Is this consistent with the

24   chemistry of the compound (i.e. solubility)? Is it consistent with the

25   postulated effects of this compound on spermatozoa?

26

27   i)   *Recommendations regarding specific statements include*:

1   i)   *Pages 5-1, lines 29-31*

2   Reword this sentence to make it neutral. "Because the results were

3   not analyzed statistically and other details regarding the duration of

4   the mating periods were not present, it is not possible to conclude

5   that 1,3-butadiene either had or did not have an effect on fertility in

6   rats."

7

8   ii)   *Pages 5-9, lines 27-29*

9   Under what condition is 108± ppm 4 vinyl-1-cyclohexene

10   identified? What is the stock solution? The missing information

11   must be added.

12

13   iii) *Pages 5-25, tables 5-13*

14   Why are only some of the studies of structurally related

15   compounds listed on this table? Why are negative observations not

16   included? The explanation given on pp.5-24, lines 17-19 was weak.

17   Since neoplastic lesions in reproductive organs were seen, these

18   findings should be added here, albeit identified as neoplastic or non

19   neoplastic. The title of this table does not limit effects on neoplastic

20   status. However, the table should not just give positive studies but

21   also include negative observations, like those in rats. The

22   observations of decreased Graafian follicules should be included if

23   significant (note significance status was not given in lines 25-27).

24

25   iv)   *Pages 5-28, lines 15-17*

26   The summary introduces new structure-activity information from

27   Maronpot, 1987. This data should be introduced and integrated

1   with Chapter 5 prior to the summary. See also summary lines

2   7-33.

3

4   v)   *Pages 5-28*

5   The summary speculates about reduced steroidogenesis, has any

6   study measured steroid levels?

7

8   vi)   *Pages 5-28, lines 32-33*

9   The Committee did not see any data presented to support this

10   speculation, in fact the observed effects of 1,3-butadiene on

11   spermatozoa and spermatids (discussed in the next paragraph)

12   argues against this "protective" effect of this barrier. The

13   Committee recommends the removal of this sentence.

14

15   vii) *Table 5-1*

16   Table 5-1 has numbers in parenthesis that are not defined. The

17   numbers that are in the parenthesis need to be listed in the footnote.

18

19   viii) *Section 5.1.6*

20   Sections such as this would be clearer if a data table would

21   accompany the text. For example, see Tables 5-4 through 5-12.

22

23   ix)   *Page 5-28, lines 11-17*

24   The paragraph on ovarian lesions fails to present the well

25   established mechanism for ovarian toxicity and carcinogenicity.

26   DEB is highly toxic to the ovary, which makes it non-responsive to

27   FSH. Continual elevation of FSH results in the carcinogenicity.

1

2   In summary, Chapter 5 does not prepare the reader for the discussion in Chapter 9 on the

3   quantitative risk assessment for 1,3-butadiene. For example, how does the biology information

4   affect your choice of models? In Chapter 5, the Agency briefly makes a statement about

5   thresholds, but does not explain implications for quantitative risk assessment. How did the

6   Agency decide to drop the highest dose levels, was this because of excessive toxicity? If so, the

7   EPA should explain the rationale. What criteria will the EPA apply to let he reader know when

8   the Agency will drop the doses?

9

10   **3.1.6 Chapter 6 - Toxicity in Animals**

11   This chapter provides detailed information about subchronic, chronic and carcinogenicity

12   studies published from 1985 to present. Only three studies are mentioned in the subchronic

13   section. It is unclear why other repeat dose studies are not reviewed. Have they **all** been

14   incorporated in other chapters? Repeat dose *in vivo* mammalian studies of 1,3 butadiene would be

15   appropriate for inclusion in this section unless it is explicitly stated that they are covered

16   elsewhere in the document. It is also important to insure that the data from *in vivo* repeat dose

17   studies get thorough review because the Inhalation Reference Concentration (RfC) value should

18   be derived on the most sensitive adverse endpoint that is meaningful for human health. The most

19   sensitive non-cancer biological effect of 1,3 butadiene currently cannot be determined from the

20   health risk assessment document, as comparative assessments are not made in any of the chapters.

21

22

23   In the chronic and carcinogenicity sections, the use of frequent text tables helps to simplify

24   and to provide clarity to the presentation. Since the chronic National Toxicology Program (NTP)

25   bioassay is a part of EPA's quantitative risk assessment, the detailed coverage is warranted. The

26   NTP study contains both chronic toxicity and carcinogenicity data and the study is discussed in

27   both areas. In order to make the chapter more understandable, the Committee recommends that

1   the Agency present the study details presented in the first section (chronic) and then refer to them

2   in the carcinogenicity section rather than repeat the material in slightly different words. As it reads

3   now, the review document gives the impression that they are different studies. The Agency should

4   also use the same categories of tumors for presenting data from the continuous treatment, 9 and

5   15-month interim sacrifices and stop-exposure study in order to permit comparisons. Most of the

6   time this was done. However, for target lymphatic tumors the categories are different, preventing

7   a direct comparison.

8

9   A table summarizing the positive oncogenic findings across all studies specifying the dose

10   tested, and the type of tumor that was significantly elevated, should be added because of the

11   numerous organs and tumor types involved. Data from the rat oncogenicity study should be

12   included as this information is important and is only indirectly mentioned in the risk assessment.

13

14   For clarity, it would be preferable to separate the carcinogenic evidence on the mammalian

15   metabolites of 1,3 butadiene from the data on related chemicals and place it in a subsection by

16   itself. The section on related chemicals should be updated to include the results from studies on

17   styrene, isoprene and any other relevant chemicals.

18

19   At the meeting, a member of the public stated that the data in Table 6-8 is wrong and that "Poly

20   3" data should be used. The Committee feels strongly that the rat carcinogenicity data need to be

21   presented in similar detail to the NTP data. In doing so, the reader would not have to find the old

22   risk assessment to see the data.

23   The quality of the Discussion and Conclusion section is good. The observation that

24   concentration, not time is a critical determinant of potency is interesting but it is not supported by

25   a comparison of the tumor data presented in Table 6-4 and Table 6-8 from the NTP study (1993)

26   for continuous lifetime treatment and stop-exposure study. It is rare that carcinogenic data are

27   available from continuous, interim sacrifice and stop-exposure studies. Further analysis of a

1   comparison of the data from continuous and lifetime treatment would be interesting to see if there

2   is supporting evidence for a biological model of dose and time that could be used in the risk

3   assessment.

4

5   ### 3.1.7 Chapter 7 - Epidemiologic Studies of Carcinogenicity

6   It is unclear why this chapter presented details of all the historical reports for a given

7   butadiene (BD) exposed group, since more recent reports supersede the previous ones by virtue

8   of having longer follow-up and more numerous deaths. Chapter 7 should be restructured so that it

9   is a detailed report on the latest follow-up of each epidemiologic study, with perhaps any

10 additional analyses from previous reports that were not duplicated in the most recent report. More

11 specific recommendations for Chapter 7 follow.

12 a)   *Discussion of butadiene exposure and lymphosarcoma/reticulosarcoma*

13   The case for an association of butadiene exposure with

14   lymphosarcoma/reticulosarcoma (ICD 200, a no-longer-used subset of

15   non-Hodgkins lymphoma; hereafter called just "lymphosarcoma") is based on two

16   studies of butadiene monomer plants which the EPA draft report indicated had

17   excesses of lymphosarcoma. However, many of the findings did not support, or

18   called into question, the purported association, specifically:

19 i)   Texaco Study

20   The largest study group was most recently reported by Divine and

21   Hartman, 1996 and consisted of 2,795 workers with an average of 32 years

22   of follow-up (Divine and Hartman, 1996). The overall observed/expected

23   ratio (O/E) for lymphosarcoma was 9/4.7= 1.91 (95% CI= 0.87-3.6) which

24   was not statistically significant. The SMRs for those employed <5, 5-19

25   and 20+ years were 2.6, 1.8 and 0.8 respectively, which is largely counter

26   to expectations. The lymphosarcomas were concentrated among those first

27   employed during World War II (WWII)(O/E= 7/2.9= 2.4, 95%

41

1   Confidence Interval (CI) = 1.0-5.0). One feature that suggests it might be

2   a real effect is that the WW II excess was limited to those in the group with

3   jobs that entailed higher exposures ("varied exposure" group). However,

4   this elevated risk among WW II workers showed an inverse association

5   with length of employment, in spite of the fact that the authors indicate that

6   high exposures still continued into the 1950's and 1960's-- which does not

7   lend plausibility to the association. Although there was an overall excess of

8   lymphosarcoma in the group with jobs that entailed higher exposures

9   (O/E= 7/2.8= 2.5, CI= 1.0-5.1) in the Divine-Hartman study, the subset of

10 this group employed for 10+ years showed no excess (O/E= 1/1.0). Divine

11 and Hartman created an index of cumulative butadiene exposure based on a

12 job exposure matrix that considered job class and calendar time. Using a

13 time-dependent Cox regression model for cumulative exposure vs.

14 lymphosarcoma, there was not even suggestive evidence for an association

15 between exposure and risk (Relative Risk (RR)= 1.00, 95% CI= 0.97,

16 1.04). On Page 7-32, Line 6 (and also Page 11-6, Line 22), in order to

17 present a balanced summary of findings on lymphosarcoma and monomer

18 production, it should be stated here that there was no indication of an

19 exposure-response association, based on the latest follow-up by Divine and

20 Hartman (Divine and Hartman, 1996).   Two other methods of analysis

21 also reinforced the null association. Regression analyses that modeled the

22 time spent in each of their six job classes as predictors were also

23 conducted; again, there was no indication   of an association. In summary,

24 the case for an association between butadiene exposure and

25 lymphosarcoma in this study is weak.

26   The EPA report of this study either fails to note or at least to

27 consider the implications of a number of the findings noted above that go against

1   the likelihood of a causal association between butadiene and lymphosarcoma. In

2   the chapter summary (Pages 7-31 - 7-32) the Agency cites positive results from

3   earlier follow-ups of this cohort and one nominally positive result from this report

4   as their summary of the study, without noting the important findings in the study

5   that are not supportive of a positive association.

6

7   ii)   Union Carbide Study

8   Ward et al., 1996 studied 364 employees who worked at one of three units

9   that had produced butadiene monomer (Ward, et al., 1996). For

10   lymphosarcoma the ratio of O/E= 4/0.69= 5.77 (95% CI= 1.6-14.8).

11   These four cases had worked at a butadiene unit for 0.8, 2.9, 3.3 and 8.0

12   years, so the worktime of three of the four was relatively brief. Two of the

13   four, with butadiene exposures of 2.9 years and 3.3 years, had worked in

14   an acetaldehyde unit for 8 years and 29 years, and all four had exposure to

15   a variety of other chemicals at the facilities. The fact that two cases had

16   long-time exposure to acetaldehyde raises the possibility that those cases

17   may have been associated with acetaldehyde rather than butadiene, and this

18   possible confounder weakens the finding. The nature and extent of the

19   possible confounding by acetaldehyde are glossed over in the EPA 1,3-

20   butadiene report.

21

22   iii)   Shell Study

23 A third study of 614 employees at a butadiene monomer production plant

24 yielded negative results (Cowles, et.al, 1994). They were followed for an

25 average of 12 years after entry to the study (which, because of the unusual

26 criteria for study entry, was equivalent to 14-17 years of follow-up after

27 first exposure) and had an average of 7.6 years of butadiene exposure.

1   There were no deaths from lymphatic or hematopoietic cancer (1.2

2   expected). This fact was not even mentioned in the EPA report (Page

3   7-8). This cohort provides no support for an association between

4   butadiene exposure and lymphosarcoma, although the fairly short

5   follow-up time and relatively small sample size means the negative results

6   should not receive a heavy weight. Nevertheless, the EPA statement that

7   "this study failed to provide any negative evidence towards the causal

8   association" (Page 7-32, Lines 15-16; also Page 7-9, Line 22-23 and Page

9   11-7, Lines 6-8) seems to be an overstatement; the study does provide

10   some, albeit not compelling, negative evidence. In fact, it is at least

11   equivalent in size to the Ward et al study that is highlighted as providing

12   positive evidence (Ward, et.al, 1996).

13

14 iv) Delzell Study

15   A large study by Delzell et al. (1996) of 15,649 styrene-butadiene rubber

16   (SBR) workers found no association between butadiene exposure and

17   lymphosarcoma (Delzell, et al., 1996). Overall the lymphosarcoma O/E=

18   11/13.8 = 0.8 (95% CI= 0.4-1.4). In those with the greatest exposure and

19   latency (10+ years exposure and 20+ years since hire) there was no

20   elevation in lymphosarcoma risk (O/E= 4/3.9= 1.0, CI= 0.3-2.6). The

21   other SBR studies are essentially earlier versions of this study, with minor

22   differences in the cohorts, so this study summarizes the lymphosarcoma

23   results for the SBR data.

24

25   It is also of note that the coding of the ICD 200 category

26   "lymphosarcoma and reticulosarcoma," as distinct from the ICD 202

27   category of other non-Hodgkins lymphomas (NHL) for which no

1   butadiene-related excesses were found in the various studies, is very

2   unreliable. In a review of medical records for death certificates coded as

3   lymphomas (ICD 200 or 202), Matanoski et al., 1993 found that two were

4   not lymphomas at all "and the other 10 were so poorly classified into the

5   200 and 202 codes on the death certificates as compared with the hospital

6   records that we combined these ICD categories" (Page 369) (Matanoski,

7   1993). This calls into question the meaningfulness of the positive results

8   reported above which were based on death certificate diagnoses of

9   lymphosarcoma. Had the other categories of NHL (i.e., ICD 202) been

10   put with this category, it is unclear whether there would be any excesses

11   of NHL.

12

13   b)   *Discussion of butadiene exposure and leukemia risk*

14

15   Delzell et al., 1996 conducted a study of 15,649 men who had worked for

16   at least one year (during 1943-1991) at any of eight styrene-butadiene

17   rubber (SBR) plants. The study included all but one small plant of the

18   earlier Johns Hopkins study (a plant that did not begin SBR production

19   until about 1970) and updated the mortality experience at all plants

20   studied, along with conducting a much more detailed exposure

21   reconstruction for these workers (Matanoski, et.al, 1990). The cohorts

22   differed slightly because of somewhat different definitions of eligibility,

23   but, for all practical purposes, the Johns Hopkins cohort is subsumed by

24   the Delzell cohort. Thus, there is essentially only one study of SBR

25   workers, of which the Delzell, et.al, 1996 and Macaluso et al., 1996

26   reports represent the most recent follow-up-- for up to 49 years with a

27   mean of 25 years.

45

1

2   c)   *Lumping of lymphohematopoietic tumors*

3   The SAB did not feel it was appropriate to "lump" lymphohematopoietic

4   tumors. Leukemia and lymphosarcoma are separate diseases.

5

6   d)   *The Criteria for Causal Inference*

7   The Criteria for Causal Inference are biased in their presentation. They

8   should be revised to reflect the above points. It was generally agreed that

9   the SBR process meets the criteria, but the monomer does not.

10

11   e)   *Additional points that should be noted in the report*:

12   i)   In the total cohort the leukemia rate was somewhat elevated (O/E=

13   48/36.6= 1.31, 95% CI= 1.0-1.7) but the number of excess

14   leukemias was only about 11 (Delzell, 1996). Surprisingly, there

15   was no excess among those hired before 1950 (O/E= 17/16.4=

16   1.04) when one would expect the highest exposures, but there was

17   an excess among those hired during 1950-59 (O/E= 20/10.0= 2.0,

18   CI= 1.2-3.1). Research is ongoing as to whether the risk elevation

19   beginning in 1950 is a function of concomitant exposure to

20   DMDTC (Dimethyldithiocarbamate) in the SBR process beginning

21   in about 1950, but it is premature to judge that hypothesis at this

22   time.

23

24   ii)   There should be a table to present the actual exposure-response

25   data for the Delzell/Macaluso study of butadiene exposure and

26   leukemia risk, rather than just burying the values in the text (e.g.,

27   Page 7-22, Line 22 and Page 7-25, Line 7). These are among the

1		most important numbers in the report, so they should be
2		prominently displayed.
3		
4	iii)	According to Macaluso et al., 1996, the 0-dose group contained a
5		substantial number of salaried workers but the other groups
6		apparently did not. This could potentially bias the
7		exposure-response risk estimate, and it should be mentioned.
8		
9	iv)	There seems to be a substantial discrepancy between two sets of
10		risk estimates given for this study in the report on Page 7-22 (Line
11		22) and Page 7-25 (Line 7), as shown in the table below. One
12		wonders if the second set (the lower half of the table), which
13		appear to be the one used by the EPA, may be incorrect. Notice
14		that in the upper half of the table, the RR for >0-19 ppm-years is
15		1.1, whereas in the lower half, the two cells that cover the range of
16		>0-19 ppm-years have RRs of 2.0 and 2.1-- rather implausible
17		values, especially the RR of 2.0 for <1 ppm-year. The middle
18		range is likewise higher in the lower half of the table (RR=2.4 for
19		20-79, vs. 1.8 for 20-99 ppm-years in the upper half). The RRs in
20		the high exposure range also differed notably: RR=4.5 for 80+
21		ppm-years in the lower half of the table, but in the upper half the
22		RRs for 100-199 and 200+ ppm-years are 2.1 and 3.6. The reason
23		for the discrepancies is not clear; the only difference noted in the derivation
24		of the two sets of estimates is that the upper set adjusted for years
25		since hire and calendar period, whereas the lower one did not. If one
26		of these two sets is not in error, then the discrepant results suggest
27		that the results must be very sensitive to the particular cutpoints,

47

1   confounders used, etc., which would argue for observing caution so as not

2   to extrapolate from the most extreme   results. It is of note that most of

3   the analyses reported by Delzell et   al., 1996 in their technical report,

4   e.g., Tables 60-62, are similar to   the upper half of the table below,

5   rather than the lower half.

6

7   Two sets of estimates of leukemia relative risks (RR) reported

8   from the Delzell/Macaluso study in relation to butadiene exposure:

9

Exposure range (ppm-years)	0	>0-19	20 - 99	100 - 199	200+
RR	1.0	1.1	1.8	2.1	3.6
Exposure range (ppm-years)	0	<1	1 - 19	20 - 79	80+
RR	1.0	2.0	2.1	2.4	4.5

17   It is unclear from the EPA report as to how high the correlation

18   between butadiene and styrene exposure levels was in this study.

19   However, one would expect the correlation to be fairly large. (A

20   personal communication from Jeffrey Lewis indicated that the

21   correlation was 0.53.) Since there is also some indication that

22   styrene may be associated with leukemia in this study, partialling out

23   the effect that is attributable to butadiene is problematic, particularly

24   if there is more reliability or accuracy in assigning butadiene

25   exposures than in assigning styrene exposures, or vice versa, since

26   variations in reliability/accuracy could drive the proportion of

27   variance attributed to one chemical vs. the other in regression

28   analyses.

1   There is a concern about the effect of peak exposures that was not

2   considered in estimating risk. More is discussed about this issue in

3   Section f below.

4   f)   *Specific Comments*

5   i)   *Page 7-3, Line. 33*: The latency period of 10-19 years was left out.

6

7   ii)   *Page 7-5, Line. 14-15*: Singling out an intermediate subgroup that

8   gave a suggestive elevation in risk, when subgroups with more

9   exposure did not, is a questionable scientific procedure (i.e., picking

10   and choosing the ad hoc results that support a particular point of

11   view).

12

13   iii) *Page. 7-20, Line. 1-5 & Page. 7-33, Line. 9-12*: It seems curious to

14   report subgroup analyses based on just 3 of the 8 plants, especially

15   when the reason given for choosing them ("three plants who had

16   geometric means of exposure" out of the 7 plants with

17   measurements) seems irrelevant.

18

19   iv)   *Page 7-21, Line 30-32*: This says, "When this subcohort was

20   further restricted..." Please define the subcohort that is discussed,

21   and also state the endpoint that is being discussed.

22

23   v)   *Page 7-23, Line 25-26*: One important feature was that the job-

24   exposure matrices (JEM) estimates were specific for calendar time.

25   This should be noted.

26

27   vi)   *Page 7-31, Line 32-33*: It is inappropriate to cite positive findings

1   from some earlier follow-up of the cohort when these were not

2   confirmed by the latest follow-up.

3

4   vii)   *Page 7-33, Line 1-8 and Page 11-5, Line 12-25*: In order to

5   present a balanced review of the Matanoski case-control study, the

6   re-analysis by Cole et al., 1993 that yielded a null RR should also be

7   reported, with an indication of how highly sensitive the results were

8   to the particular cutpoints chosen (Acquavella, et al., 1994). The

9   Cole-Acquavella results are mentioned only obliquely, and the

10   discrepancy is not articulated and its implication are not considered.

11

12   viii)   *Page 7-34, Line 33,35*: To be more balanced, the report should

13   indicate that the Meinhardt results were not statistically significant.

14

15   ix)   *Page 7-36, Line 23:* This sentence should be deleted because it

16   suggests that the stop-exposure studies conducted by Melnick et al.

17   (1990) confirm the findings of excess lymphosarcoma among

18   short-term monomer workers in the Divine et al. (1996) study. This

19   statement is incorrect.

20

21   x)   *Page 7-36, Line 23:* There is no way that the stop exposure

22   studied in mice "confirm" the short term worker effect. Long term

23   workers employed at the same time and later did not develop

24   increased lymphosarcomas.

25

26   **3.1.8 Chapter 8 - Pharmacokinetic Modeling**

27

1   There was consensus that this chapter is out of date. The chapter should be updated and

2   should include the most recently published PBPK models. A major data gap is the lack of

3   modeling of 3,4-epoxy-1,2-butanediol (EBD). A model describing the kinetics of this metabolite

4   would be useful in relating external exposure concentrations to measures of internal dosimetry

5   such as hemoglobin adducts. Such a linkage would facilitate reconstruction of BD exposure

6   profile in exposed humans. A majority of the members were of the opinion that since DEB is very

7   likely involved in BD-induced carcinogenesis, current models which describe this metabolite

8   would be adequate for risk assessment. Other members felt that other metabolites, such as the

9   diol, may play an important role in carcinogenesis at some sites, and that these metabolites needed

10   to be addressed.

11

12   The technical quality and comprehensiveness of this chapter could be improved greatly by

13   the inclusion of the most recent relevant literature on butadiene toxicokinetic modeling. Specific

14   suggestions regarding a reorganization of Chapter 8 are offered below. Chapter 8 of the draft

15   document on toxicokinetic modeling does not reflect the current state of knowledge regarding

16   PBPK models for butadiene. Rather, this chapter describes and critiques the initial attempts of

17   several laboratories to independently develop "first-generation" PBPK models for butadiene.

18   These models were developed often without benefit of critical data such as solubility parameters

19   and metabolic rate parameters. Additionally, definitive data on concentrations of butadiene and its

20   metabolites in tissues of animals exposed to butadiene were largely lacking, precluding rigorous

21   model validation. As such, some of the conclusions that the investigators drew from these early

22   models are no longer relevant. It would be more appropriate to briefly acknowledge these early

23   and important contributions. Then, attention could be focused on a serious critique of the more

24   recent PBPK models (Sweeney et al, 1996, Csanady et al, 1996, Reitz et al, 1996, and Sweeney et

25   al, 1997). These later models represent a significant advance over the previous generation PBPK

26   models in that they describe the kinetics of diepoxybutane, a critical metabolite of 1,3-butadiene.

27   Inclusion of a discussion of state-of-the-art models for butadiene kinetics would

1   significantly strengthen the scientific quality of the document. In particular, the numerous

2   statements throughout the document indicating that an adequate PBPK model for butadiene risk

3   assessment is not available should be revised to reflect the current state-of-the-art. At least two

4   models that describe the kinetics of butadiene, epoxybutene and diepoxybutane were available to

5   the EPA in 1996 (Csanady et al, 1996, Reitz et al, 1996) and one was published in 1997 (Sweeney

6   et al, 1997). A majority of the Committee felt that any one of these models, combined with

7   available in vitro metabolic rate constants could be used to obtain more refined human dose

8   estimates while other Committee members were concerned about the degree to which some

9   models were able to describe the available data, the extent to which parameters had to be changed

10   from measured values to obtain reasonable fits and the accuracy of the low dose extrapolation. As

11   noted by the Agency, these refined dose estimates could be used in the animal based risk

12   assessments for both cancer and reproductive toxicology endpoints. In accord with the revised

13   EPA Cancer Risk Assessment Guidelines and given what is understood regarding the mechanisms

14   of butadiene toxicity, a more scientifically based risk estimate for environmental exposure to

15   butadiene is the most appropriate course of action. In chapter 8, concern is expressed that serious

16   uncertainties exist pertaining to the model structure, parameter values and validation for the

17   various PBPK models. It is true that each of the models differ somewhat in the details and that the

18   parameter values chosen by various investigators are similar but not identical. However, these

19   models are similar enough that any of the models is capable of predicting blood concentrations of

20   butadiene, epoxybutene, and diepoxybutane following inhalation exposure to butadiene. This

21   suggests that whatever differences in the underlying model structure exist, these differences are

22   minor. Most importantly, each of these second generation PBPK models fully describes the

23   kinetics of diepoxybutane. A majority of the Committee felt that since it is likely that it is

24   diepoxybutane that is the critical metabolite for initiation of the carcinogenic effects following

25   exposure to butadiene, inclusion of this metabolite makes these models especially useful for risk

26   assessment. An alternative view on the Committee was that other metabolites, such as the diol,

27   may play an important role and should be addressed in developing models predictive of human

1   risk. Also, the models of the diepoxide require validation and further development to address

2   parameter and structural uncertainty as well as interindividual variability in human metabolism.

3

4   The conclusions drawn in Chapter 8 need to be revisited in light of information on the

5   kinetics and PBPK modeling of butadiene that is not presented in the draft report. In the

6   conclusions, the Agency notes five areas in which more research is needed including:

7

8   a)   evaluation of diepoxybutane kinetics,

9

10   b)   investigation of the validity of in vitro metabolic data for extrapolating in vivo

11   exposure,

12

13   c)   clarification of values of various physiological parameters,

14

15   d)   better characterization of the distribution values for the human metabolic rates, and

16

17

18   e)   more measurement of tissue concentrations of metabolites for model validation.

19

20   Comments on each of these areas are noted below:

21   a)   *Evaluation of diepoxybutane kinetics*

22   Revision of the draft risk assessment to include data published since January 31,

23   1997 will resolve this need. A paper by Valentine et al (1997) reports on the

24   pharmacokinetics of diepoxybutane in rats after IV injection, the most appropriate

25   route of administration for this non-volatile chemical. Sweeney et al, (1997) were

26   able to successfully simulate this data using a PBPK model developed for butadiene,

27   epoxybutene, and diepoxybutane. Given the high mutagenic potency   of

1 diepoxybutane, it is unlikely that kinetic measurements of this chemical will ever be

2 made in humans in vivo. However, parallel studies using lung and liver tissue

3 samples have quantitated the rates of hydrolysis and glutathione   conjugation of

4 diepoxybutane in rats, mice and humans (Boogaard, et al. 1996 and Boogaard and

5 Bond 1996) and in vitro studies on the oxidation of epoxybutene have characterized

6 the rate of diepoxybutane formation (Seaton, et al., 1995).

7

8 b) *Investigation of the validity of in vitro metabolic data for extrapolating in vivo*

9 *exposure*.

10 The validity of the in vitro -in vivo extrapolation for butadiene has been investigated

11 in a manuscript by Sweeney, et al.(1997), in which the in vitro data describing the

12 rates of butadiene oxidation, epoxybutene oxidation, and hydrolysis and glutathione

13 conjugation of epoxybutene and diepoxybutane determined in   vitro were used

14 directly in a PBPK model and the model predictions were   validated against in vivo

15 data obtained from butadiene exposure of mice and rats. The ability of the model to

16 simulate both epoxybutene and diepoxybutane blood and tissue concentrations

17 following exposure to butadiene points to the usefulness of in vitro parameters in in

18 vivo models. For carcinogenic or potentially carcinogenic chemicals it is unlikely

19 that direct toxicokinetic measurements will ever be made in people. Therefore, the

20 only viable option is to conduct in vitro experiments in which metabolic rates are

21 determined in human and animal tissue samples and then use these metabolic rates in

22 the context of the PBPK model to predict blood and tissue concentrations of both

23 animal species and humans. Acceptance of this in vitro -in vivo extrapolation

24 strategy would serve to   encourage the collection of appropriate data with the

25 ultimate goal of   incorporating more mechanistic information into the risk

26 assessment process.

27

1   c)   *Clarification of values of various physiological parameters*

2   Regarding the choice of model parameters, there is undue concern that all

3   investigators have not used the same values for physiological parameters. All

4   investigators developing butadiene PBPK models have selected specific parameter

5   values from a distribution of widely accepted parameters values for various

6   physiological parameters in these models. Selection of a single value for ventilation,

7   perfusion, blood flow or organ volume is in itself a simplification of the biological

8   system being modeled. Many of these parameters vary for a single individual

9   throughout the day. All of these parameters vary among individuals.   The important

10   point is that the selected values are physiologically realistic.

11

12   d)   *Better characterization of the distribution values for the human metabolic rates*

13   This statement comes across as a gratuitous "more research is needed." As noted

14   previously, V_{max} and K_m values have been determined for a number of human

15   samples. The fact is that the data base for butadiene is particularly robust in terms

16   of the number of samples characterized. If the EPA has concerns regarding the

17   quantitative and qualitative distribution of human metabolic rates, these concerns

18   should be stated much more explicitly and in sufficient detail that specific research

19   could be conducted to meet this need.

20

21

22   e)   *More measurement of tissue concentrations of metabolites for model validation*

23   Over 13 individual inhalation exposures of mice and rats and monkeys to various

24   concentrations of butadiene have been conducted. Measurements at steady state

25   and post exposure for butadiene and its metabolites have been made in blood and

26   tissues. These data have been summarized by Himmelstein et al (1997). The data

27   are remarkably consistent across laboratories and consistently point to dramatic

1   species differences in metabolism of butadiene. It is really not clear what additional

2   data the Agency might find useful for model validation.

3

4   Specific comments on Chapter 8 follow:

5   a)   *Page 8-1, lines 6-7.* The PBPK models cited by the EPA in this report should be

6   updated. More mechanistic and sophisticated models are now available including

7   Reitz, et al. (1996), Csanady, et al. (1996), Sweeney, et al. (1996), Sweeney, et al.

8   (1997), and Kohn (1997).

9

10   b)   *Page 8-2, lines 1-32.* The model of Hattis and Wasson (1987) was developed

11   before there was any experimental data on the rates of 1,3-butadiene activation and

12   detoxication in experimental animals and before there was any experimentally

13   determined data for partition coefficients, estimates of solubility that are used in

14   PBPK models. Given the acknowledged limitation of this model, it would be   more

15   appropriate to simply mention that this model is one of the first PBPK models to be

16   developed without going into details regarding the model   predictions. This is

17   particularly important given the conclusion of Hattis and Wasson that "differences in

18   pharmacokinetics failed to account for differences in carcinogenesis between mice

19   and rats and that with respect to risk assessment, uncertainties in PBPK modeling

20   are trivial compared with the differences in apparent sensitivities between these

21   species" (Page 8-2, lines 18-32). The overwhelming body of experimental data on

22   the toxicokinetics of 1,3-butadiene and its metabolites collected in rats and mice

23   since the publication of the Hattis   and Wasson model have shown that differences

24   in pharmacokinetics between   these two species can account for species differences

25   in carcinogenesis. It is also significant that the Hattis and Wasson model was an

26   unpublished report and never benefited from peer-review. It would be more

27   appropriate for the EPA to devote a paragraph to the presentation of the Hattis and

1		Wasson model similar to what was done for the Hallenbeck (1992) model (page 8-4,
2		lines 3-9).
3		
4	c)	*Page 8-4, lines 10-33.* It might be appropriate for the EPA to cite the Kohn
5		(1997) model instead of the 1993 model primarily because the model of Kohn and
6		Melnick (1993) relied on theoretically derived partition coefficients rather than
7		experimentally determined partition coefficients. The use of these calculated
8		partition coefficients resulted in overpredictions of the concentrations of
9		1,3-butadiene in tissues, especially fat.
10		
11	d)	*Page 8-7, lines 27-34.* As noted above, the use of empirically derived calculated
12		partition coefficients rather than experimentally determined values led Kohn and
13		Melnick in their 1993 model to conclude that storage in fat is a significant fraction
14		of the retained 1,3-butadiene, especially in rats and humans. It is generally
15		recognized that this conclusion is based on the use of calculated rather than
16		experimentally measured partition coefficients. Inclusion of this paragraph in the
17		chapter may lead readers not knowledgeable in 1,3-butadiene toxicokinetics to
18		believe that this model prediction is accurate. While the EPA does discuss this
19		limitation (page 8-8, lines 1-13), it is still misleading for the EPA to devote
20		significant discussion to models in which theoretical values are used when later
21		experiments determined the values to be inaccurate. It would be much more
22		prudent for the EPA to report the most recent PBPK models noting when
23		necessary that these laboratories had also participated in the development of earlier
24		models.
25		
26	e)	*Page 8-12, lines 21-23.* The EPA notes that "Johanson and Filser are reportedly
27		working on a corresponding PBPK model for humans but it has not yet been

1   published." At this point it would be most appropriate for the EPA to cite the

2   Csanady et al (1996) PBPK model for 1,3-butadiene that includes model

3   predictions for man.

4

5   f) *Page 8-15, lines 9-11.* The EPA notes that a limitation of the model of Evelo et   al.,

6   1993 is that metabolism of butadiene is limited to the lung and the liver. This should

7   not be viewed as a limitation. The objective of PBPK models is to   account for the

8   most significant mechanistic steps in the disposition of chemicals with the goal of

9   predicting the concentration time profile of the toxic agent in either the target tissue

10   or a suitable surrogate for the target tissue such as blood. It is not practical nor is it

11   necessarily advantageous to develop a model that incorporates all pathways in the

12   disposition of a chemical however minor. The simplest models that are the most

13   useful for risk assessment are most likely to   have the greatest value since these

14   models will have the fewest number of parameters that require independent

15   experimental determination in animals and humans. Thus, it is not clear why the

16   Agency feels it is necessary in the case of butadiene to account for metabolism of

17   this chemical in all tissues of the body. Extensive modeling efforts have determined

18   that inclusion of metabolic activation in the liver, the major organ for metabolism of

19   butadiene, and in the lung, a target organ for mice, are the most appropriate from a

20   mechanistic standpoint.

21

22

23   g) *Page 8-15, line 15.* The EPA notes that the most recent PBPK model published   for

24   1,3-butadiene is the model of Medinsky et al. (1994). This statement is correct   only

25   in the context of the January 31, 1997 cutoff date for consideration of reports in this

26   document. However, the statement is misleading as there are a number of other

27   PBPK models that have been published since 1994, including models published in

1   1996 and 1997. The EPA is urged to revise this chapter to include these more recent

2   models. These models not only consider the metabolism of 1,3-butadiene, but also

3   include the disposition of its two epoxide metabolites, epoxybutene and

4   diepoxybutane, thereby making the models more appropriate for use in risk

5   assessment.

6

7 h) *Page 8-16, lines 6-14.* The EPA notes that "in the model of Medinsky et al., the

8   microsomal concentrations reported by Csanady et al. were not used to scale

9   metabolic rates. Instead, literature values for microsomal concentrations were

10   used." While the Agency correctly summarizes the approach taken, the rationale

11   for taking this approach is not appropriately presented. The objective of the study

12   reported by Csanady et al. (1992) was to determine the rates of butadiene

13   oxidation in microsomes from rodents and humans. To achieve this objective it is

14   essential that purified microsomes be obtained from liver, but it is not essential that

15   all of the microsomal protein in the liver be accounted for. Thus, Csanady et al.,

16   1992 report microsomal yield which is less than the total microsomal protein

17   content of liver or lung. Other investigators, in contrast, have sought to determine

18   the total amount of microsomal protein in liver or lung. These investigators have

19   used other techniques for this assessment. Extrapolating in vitro results expressed

20   per milligram of /microsomal protein to the entire organ requires knowledge of the

21   total amount of microsomal protein in an organ not simply the yield of microsomal

22   protein obtained in the biochemical experiment. Thus, the appropriate approach

23   for scaling in vitro rates to the whole animal is to use total microsomal total

24   protein content rather than yield. The literature values used by Medinsky et al.

25   (1994) for total microsomal protein content were similar to those used by

26   Johanson and Filser (1993) and Kohn (1997). In contrast, Kohn and Melnick

27   (1993) used the values of microsomal yield reported by Csanady et al. (1992).

1   i)   *Page 8-19, Section 8.3, Summary.* The EPA notes that "pharmacokinetic

2   modeling of 1,3-butadiene has not elucidated the reasons for interspecies

3   differences in carcinogenic response between rats and mice." This statement is not

4   true if one evaluates the current PBPK models that describe the disposition of

5   1,3-butadiene, epoxybutene and diepoxybutane. These PBPK models clearly

6   demonstrate that the dramatic species differences between rats and mice in

7   response to 1,3-butadiene are most likely related to species differences in rates of

8   formation and removal of the diepoxybutane metabolite. Experimental data and

9   PBPK model simulations indicate that mice produce far greater concentrations of

10   this reactive metabolite compared with rats. Sweeney et al. (1997) have used in

11   vitro metabolism data collected in tissues from rats, mice, and humans directly into

12   a PBPK model to make predictions regarding the epoxide concentrations in blood

13   and tissues following exposure to 1,3-butadiene. Using in vitro derived parameters

14   they were able to adequately simulate the pharmacokinetics of 1,3-butadiene,

15   epoxybutene, and diepoxybutane. This ability to use in vitro data to make in vivo

16   predictions suggests that in vitro data on rates of 1,3-butadiene, epoxybutene, and

17   diepoxybutane metabolism obtained in human tissue samples can also be used to

18   predict blood and tissue concentrations of both epoxide metabolites in humans.

19   When these simulations are conducted, using average values for human metabolic

20   rates, the results indicate that diepoxybutane concentrations in humans would be

21   orders of magnitude less than those of mice and lower than, but much more similar

22   to, concentrations predicted for rats. Thus, the EPA should revise this chapter to

23   include a discussion of the toxicokinetic models for 1,3-butadiene that are now

24   capable of simulating not only the disposition of 1,3-butadiene, but also its two

25   most important epoxide metabolites.

26

27   j)   *Page 8-19, lines 33-34.* The EPA statement that uncertainties in the existing

1   PBPK models and data make them unreliable for use in risk assessment must be

2   revisited. Given that several investigators have been able to use these models and the

3   underlying metabolic data to predict butadiene, epoxybutene, and   diepoxybutane

4   concentrations in rodents exposed to butadiene, it is not clear what additional data

5   and what uncertainties need to be resolved prior to the use of these models in risk

6   assessment. For example, Sweeney et al. (1997) have used in vitro metabolism data

7   obtained from rat, mouse, and human tissues directly in a PBPK model to make

8   predictions regarding the epoxide concentrations in blood and tissues following

9   exposure to 1,3-butadiene. Using in vitro derived parameters   they were able to

10   adequately simulate the toxicokinetics of butadiene,   epoxybutene, and

11   diepoxybutane.

12

13   k) *Page 8-20, line 5-21.* In this paragraph the EPA presents a number of criticisms

14   regarding the parameters used in the PBPK models that are not necessarily

15   accurate. For example, the EPA notes that "with respect to parameter values,

16   there are disagreements about the ventilation rate and about metabolic

17   parameters." As noted above, while it is true that each investigator uses a different

18   value for ventilation rate or metabolic parameter, the values used by all

19   investigators are within the normal range associated with these parameters. It is

20   also not unreasonable to expect that if the EPA were to use a PBPK model in risk

21   assessment that other point estimates also within the range of reported values for

22   these parameters would be chosen by the EPA.

23

24   The EPA also notes that there is a paucity of human in vitro data for

25   extension of the PPBK model to humans and that the few measurements that have been

26   made on a few metabolic parameters show a high amount of variability.   Relative to

27   other chemicals there is an extensive amount of experimental data on   the rates of

1   metabolism of 1,3-butadiene by human tissue samples. Each of the metabolic pathways

2   important in the disposition of 1,3-butadiene and its   metabolites have been

3   quantitated with V_{max} and K_m values obtained from multiple human samples. These

4   pathways include oxidation of 1,3-butadiene, oxidation of epoxybutane, hydrolysis of

5   epoxybutane, glutathione conjugation of epoxybutane, glutathione conjugation of

6   diepoxybutane, and hydrolysis of diepoxybutane.   Means and standard deviations

7   for these parameters have been calculated because multiple human samples have been

8   used. Thus, sample distributions can be   generated from which population values can

9   be obtained. A PBPK model applied   to human risk assessment could employ either

10   average values to obtain   deterministic predictions or Monte Carlo simulation

11   techniques to get   probabalistic estimates of the range of responses of hundreds of

12   simulated   humans. The latter approach using Monte Carlo simulation would

13   provide some estimate of the potential variability in human response to inhaled

14   1,3-butadiene in addition to an estimate of the response of the most sensitive humans.

15   Regarding   the large amount of variability associated with these metabolic

16   parameters when measured in humans, this reflects the inherent variability in the

17   expression of   various xenobiotic metabolizing enzymes in the human population, a

18   fact that has been well documented in the literature. Thus, it is not unexpected that

19   assessment   of metabolic capacity from tissues from multiple humans should

20   yield a range of outcomes.

21

22   l)   *Page 8-20, lines 22-34.* The EPA notes that the existing models have been

23   subjected to a very limited validation mostly by comparison of simulation results

24   with chamber uptake data. This statement is true regarding the PBPK models that

25   were published in 1994 and earlier (e.g. the models reviewed in this current

26   document). However, since the publication of these first generation models,

27   multiple inhalation toxicokenetic studies have been conducted in rats and mice

1   where blood and tissue concentrations of 1,3-butadiene, epoxybutene, and

2   diepoxybutane have been quantitated following inhalation exposure to

3   1,3-butadiene. The second generation models that include the prediction of not

4   only epoxybutene but also diepoxybutane have utilized this recent in vivo

5   toxicokenetic data for model validation.

6   The EPA also notes that "for PBPK models to be more reliable, they

7   should also be validated against tissue concentration data for various metabolites

8   and various tissues. More recently these data have become available although they

9   must be interpreted with caution because it appears that metabolites in some   of

10   the tissues are subject to further metabolism during the lag time between

11   termination of exposure and measurement of tissue concentrations." This statement

12   is true and ironically the implications of post exposure metabolism were first

13   recognized when one of these second generation PBPK models (Sweeney et al.,

14   1996) failed to adequately simulate tissue concentrations of epoxide metabolites.

15   When the authors modified the initial conditions of the model to reflect the time

16   lag between termination of exposure and measurement of tissue concentrations and

17   the capacity of the tissues to metabolize 1,3-butadiene post exposure, they were

18   successful in predicting the actual measured epoxide tissue concentrations.

19

20   m)   *Page 8-21, lines 4-23.* The EPA concludes that the existing PBPK models and

21   data cannot explain the interspecies differences in 1,3-butadiene carcinogenicity.

22   As noted previously, the first PBPK models discussed in this report did not include

23   the toxicokinetics of diepoxybutane. More recent second generation PBPK models

24   that include the formation and elimination of this metabolite are successful in

25   simulating in vivo data in both rats and mice for 1,3-butadiene for epoxybutene and

26   diepoxybutane concentrations in blood and tissues. Both model predictions and

27   experimental data indicate that the dramatic interspecies differences in carcinogenic

1   response to 1,3-butadiene can, in fact, be explained by the dramatic interspecies

2   differences in circulating concentrations of the diepoxybutane.

3

4   **3.1.9 Chapter 9 - Quantitative Risk Assessment for 1,3-Butadiene**

5

6   The Agency is to be commended for its efforts to develop a clear, well-reasoned

7   quantitative assessment of 1,3-butadiene cancer risk . The assessment is made particularly

8   difficult, by the continuing release of new and relevant scientific data, including toxicokinetic,

9   epidemiological and mechanistic data. Furthermore the analyses were undertaken after the release

10   and SAB review of the proposed EPA carcinogen guidelines but before they have been finalized.

11   The Agency is to be commended for looking at new approaches, such as the benchmark dose

12   procedures, to improve quantitative assessment on non-cancer endpoints. However, the

13   Committee has submitted suggestions on how to further improve these approaches and how to

14   make these new approaches more clear, accurate and consistent. Specific comments on chapter 9

15   are given below.

16

17   a)   *Dose response analysis of the human carcinogenesis data*

18

19   *i) Quality of human data*

20

21   The Committee agrees that the Agency's risk assessment of 1,3-   butadiene

22   should include the latest follow-up of each of the epidemiologic studies, including

23   Delzell et al. (1995) and Matanoski (1997). Delzell fits a variety of   dose response

24   models, accommodating a wide range of dose response curves. The models fit equally

25   well, and as noted by EPA as a whole were consistent with a considerable range of low

26   dose risk predictions. Thus the Delzell study does not permit one to discriminate

27   among models for the purpose of low dose prediction.   In this sense the data can be

1   viewed as limited for the purpose of low dose risk prediction. The reason why

2   equivalent model fits are obtained requires further explanation in order to convey a

3   greater appreciation of the nature of the dose response data. For example, is this due

4   to a relatively narrow range of dose   response data in the exposed cohort or to

5   considerable scatter?

6

7   The assessment notes that few subjects were exposed to benzene, but

8   benzene was not seen to confound the relationship between butadiene or styrene

9   and leukemia. Because benzene is widely recognized as a human leukemiogen,

10   further discussion of this point is needed (e.g., an indication of whether the

11   benzene exposures were insufficient to cause effects; consistency with expectations

12   based on a Chinese cohort).

13

14   In the section on uncertainties, the potential importance of exposure

15   misclassification in the Delzell study is discussed, but further discussion of the

16   uncertainties of dose reconstruction and the potential magnitude of impact on the

17   risk predictions is desirable.

18

19   *ii) Presentation of multiple model fits and low dose extrapolations*

20

21   The results of the multiple model fits derived by Delzell and colleagues   are

22   tabulated, along with low dose risk predictions. While it is reasonable to   compare

23   results to make the point that the data do not provide the basis for discriminating

24   among models of quite different shapes, once it is established that   this is the case the

25   further presentation of low dose analyses is not needed and unnecessarily complicates

26   the presentation (e.g., in Table 9-3 and Table 9-4). Presentation of results for the linear

27   model would suffice, along with perhaps   effect concentration estimates, within but

1   not outside the range of observation.   The other point to be made, that the

2   exposure concentration at 1% risk level ($EC_{01)}$ results for the final versus initial square

3   root model differ by roughly 40 - 50%   could also be made, for example, in the text.

4   It motivates the need for obtaining comparable estimates for the linear model, by

5   omitting styrene and race (as was   done for the "final" square root model).

6

7   Included are the results for the square root model, as well as the power

8   model, which is apparently best fit by a coefficient less than unity. Superlinear

9   models such as these are not biologically plausible, and should therefore not be

10   used for low dose risk predictions. Synergy between genotoxicity and cell

11   proliferation frequently occurs as does saturation of detoxification and metabolic

12   activation. These factors result in sublinear data sets. Metabolic data for butadiene

13   clearly show saturation of activation and the molecular dosimetry data are

14   supralinear. An analogous point was made by the Committee in commenting on

15   proposed EPA carcinogen guidelines regarding the use of the Weibull-in-dose

16   model without constraints for exponents less than unity for risk prediction. Lack of

17   biological plausibility is another reason for not presenting low dose risk predictions

18   for the square root and power models.

19

20   The measure of deviance for the models seem to be within 0.4, while

21   normally one would say models differ if their difference in deviance is about 4.0.

22   Also, the best fit for the multiplicative model is sublinear; for the power model it is

23   superlinear and as noted by Delzell and colleagues, the square root model fits best

24   (although marginally so) - again, this reinforces the notion that the data provide

25   limited information on dose response. Perhaps replacing the dose response figures

26   with one figure showing the data points with their confidence intervals, and

27   superimposing on the figure the various models fit would provide a better

1   understanding of why this is the case. The inclusion of data should clearly illustrate

2   why the data are equally explained by the wide range of models tried.

3

4   *iii)   Dose rate, duration and timing*

5

6   With benzene induced leukemia and lymphoma the recency of exposure is

7   an important feature of the dose -time-response, with recent exposures most

8   important for leukemia, and distant, high exposure most important for

9   lymphosarcoma. On Page 9-2, the assessment notes the finding of Delzell and

10   colleagues that excluding exposures within 5-10 years of death slightly increased

11   the exposure response relationship but excluding exposures within the last 20 years

12   almost eliminated the relationship. It would be desirable for the Agency to explore

13   in detail the possible dose time relationships. On a related point, Delzell and

14   colleagues have recently reported on the possible importance of peak exposures to

15   1,3-butadiene on leukemia risk (Delzell, et al., 1995). It is important to explore the

16   dose rate/time/response issues through careful analyses of the Delzell data set.

17

18   *iv)   Extrapolation of occupational results to general population*

19

20   The available data for risk assessment is for males, exposed as young and

21   middle-aged adults, and for a selected group in that they were workers in the jobs

22   studied. The extent to which this group can be used to represent the general

23   population should be addressed more carefully, with an attempt to quantitatively

24   address, where possible, differences between cancer potency for the occupationally

25   exposed and the general population. Such an analysis should consider the

26   following:

27

a)   *potential increased sensitivity for women and girls*

No dose response information appears to be available for women. The mammary gland was a sensitive site in both rats and mice. Given the large public health concern for breast cancer in women, and the findings in the bioassay, the issue should be explicitly addressed. In terms of overall risk, female rats in the Hazelton 1981 study were clearly more sensitive than male rat, at least by an order of magnitude. Although given the uncertainties in the quantitative analysis, this is less clear for the mouse, cancer potency estimates were greater in the female compared to the male by about a factor of 2. Although toxicokinetic differences are not apparent in hemoglobin data for men and women with similar exposure, the potential for increased susceptibility of mammary tissue cannot be excluded.

b)   *healthy worker effect*

The extent to which a presumably small adjustment may be appropriate should addressed. The extent to which restrictions on smoking in occupational settings with butadiene exposure may   have contributed to the healthy worker effect should be addressed   by the Agency. There was a diversity of opinion amongst the Committee on this issue. Internal comparisons done by Delzell et   al. (1995) that were used to derive the human cancer potency estimate are not influenced by the healthy worker effect since no external comparison population is being used. However, the   healthy worker effect is an important consideration in extrapolating from the occupational setting to the

1   general population, and this   could not be assessed through an internal

2   comparison.

3

4   c)   *exposure during a working life versus other life stages*

5   Issues falling under this category to address include the potential

6   for inherent increased susceptibilities at different ages (e.g., *in*

7   *utero*, and during infancy, childhood and old age), and those related

8   to the stages and mechanisms of carcinogenesis (e.g., time of

9   exposure versus observation; after a point, diminution of risk with

10   time since exposure). Analyses of the Delzell and perhaps

11   Matanowski et al. 1997 data, and observations from the large

12   Chinese benzene/leukemia cohort may shed light on the second

13   issue. The available data on butadiene obviously does not provide

14   direct information on the first issue, but data on leukemogenesis

15   from other agents may.

16

17

18   d)   *other potentially susceptible subpopulations*

19   The potential range of susceptibility within the general population

20   should be explored quantitatively, by for example, considering the

21   impact of polymorphisms on low dose risk, and addressing the   extent

22   to which certain potentially susceptible groups are contained within the

23   Delzell cohort.

24

25   The document would be improved by presenting greater detail on the derivation of lifetime

26   risks from continuous exposure from the fits to the occupational data. Perhaps this could

27   be done in an appendix.

1   *v)   Choice of maximum likelihood estimate over Upper Confidence Limit*

2   *(UCL) on potency/LEC*(95% lower confidence interval)

3

4   Maximum likelihood estimates (MLEs) rather than lower confidence   bound

5   on the effective concentration (EC) (or upper confidence bound on   potency) are

6   used in the final characterization of the dose response derived from the human data.

7   It has been the practice of the Agency to use maximum   likelihood estimates in

8   potency derivations from human data and upper   confidence bounds on derivations

9   from animal data. The rationale provided in the 1,3-butadiene document is that the

10   simple linear model fit to the human data does not have the instabilities that can be

11   associated with polynomial fits, and that there is far less uncertainty in the potency

12   estimate derived from human data. In this particular case there are a number of

13   unaddressed issues suggesting that, at least, the EC_{01} may be underestimated for the

14   general population. These include   exposure misclassification in the Delzell cohort,

15   the lack of availability of data on all but males exposed occupationally during

16   adulthood, and the lack of   availability of a fit of a "final" linear model (comparable

17   to the model for the square root model [omitting styrene and race]). In addition, as

18   previously stated   in this report, there is a possibility that there was an

19   overestimation due to exposures being underestimated in the Delzell et al. (1995)

20   study. It would be preferable to explicitly take these factors into account, to the

21   extent possible; it is unlikely that application of a confidence bound will provide

22   adequate correction.

23

24   *vi)   Availability of Delzell study*

25

26   Although the Delzell study has been published (Delzell, et al., 1996;

27   Macaluso, et al., 1996), some of the key analyses that aid in interpreting the data

1   are available only in an unpublished technical report and thus is of limited availability

2   to the public. At the meeting the Committee heard that there was peer review of the

3   unpublished technical report . A report so critical to the dose   response analysis and

4   so heavily cited in the document should be made widely available to the public, along

5   with description of the peer review conducted by the Agency.

6

7

8   b)   *Dose response analysis of animal cancer bioassay data*

9

10   i)   *Time dependent analyses*

11

12   The default, time dependent analyses of the cancer bioassay data were

13   carefully done and are well presented.

14

15   Because the cancer incidence rates are large for some tumor sites, errors

16   are introduced if it is assumed the polynomial represents a true model of multistage

17   carcinogenesis, as Suresh Moolgavkar (1994) has pointed out (Moolgavkar,

18   1994). This should be acknowledged in discussions which infer number of stages

19   on the basis of modeling results (e.g., Page 9-20). The errors may be relatively

20   small given the availability of data at relatively low doses; it would be desirable to

21   attempt to gain an understanding of the magnitude of the error for these fits.

22

23   The method used to develop the upper 95% confidence bound for the sum

24   of the incremental lifetime unit cancer risk for humans (q_1) parameter across sites is

25   reasonable. An alternative approach for future assessments would be to derive the

26   distribution for the q_1 parameter for each tumor site and use Monte Carlo

27   simulation or numerical techniques to obtain the distribution of the sum. The result

1   should differ marginally from the one presented, and thus it is not been suggested

2   that the analysis be redone for this case.

3

4   *ii)*   *Lack of site concordance across species*

5

6   While leukemia is the observed endpoint in humans, leukemias are not

7   observed in the animal bioassay in either rats or mice, in either sex. The rationale

8   for the default inferences regarding site concordance, and the inclusion of sites

9   such as the Harderian gland and forestomach should be clearly stated. Ovarian

10   tumors are observed, along with ovarian atrophy in the animal bioassay. The

11   degree to which the toxicity may have played a role in certain sites observed in the

12   bioassay and the extent of relevance to humans exposed at lower doses should be

13   discussed.

14

15

16   *iii) Toxicokinetics*

17

18   A variety of viewpoints were expressed within the Committee over the extent to

19   which toxicokinetic analyses should be incorporated into the assessment for use in

20   interspecies and high to low dose extrapolation. The Agency was criticized by some for

21   not incorporating the results of the recent models. Others noted significant deficiencies in

22   the proposed models and the very recent developments suggesting the field was

23   undergoing rapid development, and found the models speculative. Nonetheless, the

24   different toxicokinetic hypotheses and the hypothesized role of the various metabolites

25   should be discussed, and at least qualitatively, with an indication of the degree to which

26   the assessment would be impacted if some of the hypotheses were later proven true. The

27   discussion should acknowledge the variety of competing viewpoints and hypotheses of

1   researchers in the field. Where information on toxicokinetics and mechanism provide

2   adequate understanding of findings at certain sites within one rodent and not the other,

3   this should be given.

4

5

6   c)   *The assessment of the developmental and reproductive toxicity endpoints*

7

8   Improvement in non cancer risk assessment, such as use of effect levels as suggested in the

9   benchmark dose procedures, was supported by the Committee. However, since these are

10   relatively new procedures, the Agency must meet a high standard of clarity and transparency in

11   their initial applications as presented in this 1,3-butadiene assessment. The Committee encourages

12   the EPA to review the accuracy of their calculations. The Committee also requests discussion in

13   the risk assessment EPA to explain the use of their newly proposed models, especially those

14   modeling time to impact and to provide additional explanation of the safety factors applied to the

15   benchmark calculations resulting from their modeling exercises:

16

17   i)   A clear rationale for the selection of one particular point of departure over another

18   is needed -- for each case for which the default is applied and for cases deviating

19   from the default.

20

21   ii)   Where it is not obvious the biological significance of particular endpoints for the

22   assessment should be conveyed.

23

24   iii)   When applying novel approaches, such as the time-to-response modeling of

25   atrophy, the advantages and limitations in utilizing the approach in the context of

26   reference concentration estimation should be clearly understood and presented.

27

1   iv)   The incorporation of information on toxicokinetics (e.g., ovotoxicity of diepoxide)

2   should be used where possible on at least a qualitative if not quantitative basis to

3   inform the choice of endpoints, modeling and uncertainty factors.

4   v)   The exclusion of points at high doses contributing to a poor fit is a reasonable

5   approach, and typically can be explained, for example, in terms of toxicities other than

6   the one being modeled, or toxicokinetics. When the approach is applied   where

7   possible the specific rationale for the case at hand should be presented. The   use of the

8   procedure and the rationale provided for should be consistent across endpoints and

9   across assessments for the same scenarios.

10

11   vi)   There may be a difference of opinion among the EHC about the appropriateness of

12   using a risk reduction factor, when reference concentrations are based on

13   benchmarks associated with risk of effect. The rationale for use of this factor needs

14   to be clearly laid out.

15

16   vii)   Analyses of this type applied to these endpoints may be unfamiliar to others

17   working in the general area. Confusion over the presentation of some results

18   (e.g., logarithm versus linear scales) was obvious in some of the comments

19   received. Also, calculations should be easily followed. Some of the Committee

20   noted, as did several consultants presenting comments to the Committee, that

21   mathematical errors were evident. EPA should carefully review this section of the

22   document. Tables and figures should be free standing, with statistics referenced.

23

24   Additional comments recommendations for Chapter 9 include the following:

25   a)   *Page 9-4*: Since previous EPA lifetime risk estimates have used a 70-year

26   time-frame, it would seem appropriate to follow this precedent for the sake of

27   comparability, rather than use an 85-year estimate.

1   b)   *Figures 9-9 to 9-14:* These figures should be prepared so that the axis are

2   understandable without reading the full text.

3   c)   *Page 9-27, line 33:* Why does this sentence say 600 ppm and above? Why doesn't

4   it say 625 ppm, a dose that was tested?

5

6   d)   *Page 9-5 & 9-7:* There is a discrepancy in the 95% lower confidence interval with

7   a 1% level of risk, LEC_1, value between the text and the figure (0.12 ppm vs.

8   0.066 ppm). Two of the figures appear to be mislabeled.

9

10   e)   *Page 9-13, lines 18-19:* It was difficult to follow the logic in the last sentence.

11   This sentence should be modified to provide necessary rationale to follow this

12   choice.

13

14

15   f)   *Page 9-36, lines 13,14:* What is the rationale for this statement? Either the

16   explanation should be expanded or the statement should be deleted.

17

18   g)   *Tables 9-13 thru 9-15:* Statistics should be included for these summary tables.

19

20   h)   *Figures 9-9 thru 9-14:* For clarity, the axis should be labeled so that reader can

21   easily convert dose to ppm without going back to the text. Also figures, like tables,

22   should indicate when exposures were adjusted to 24 hour daily exposures.

23

24   i)   *Section 9.3:* Although the 95% lower confidence interval with a 10% level of risk

25   (LEC_{10}) values are taken for the "point of departure" for the RfC's calculated for

26   the benchmark based approach, minimal discussion was given as to why the LEC_{10}

27   was chosen over the 95% lower confidence interval with a 5% level of risk (LEC_5)

1   nor why the 95% lower confidence interval with a x% level of risk (LEC_x) versus ED_x

2   values are chosen. Justification and rationale for this issue for all modeled endpoints

3   should be provided.

4   j)   *Pages 9-49, lines 9-13 and Table 9-21:* The Committee was unable to follow the

5   logic in the use of the "risk reduction factor" that was applied to the benchmark

6   dose based RfC. This section should be rewritten so that it is comprehensible.

7

8   k)   *Pages 9-49, lines 33-34*: Is this statement true for dominant lethal effects as well

9   as fetal weight reduction? This Committee urges caution in such general

10   statements or authors should provide specific justification of these statements.

11

12   l)   *Pages 9-42, lines 5-7:* Text cites Allen et al. 1994b as source of information

13   supporting the use of LEC_{10} as being "at or below the range of detectable

14   responses." (Allen, 1994) Since this paper dealt only with developmental toxicity

15   data, this statement as well as those later in this section are extrapolations from

16   that research. The following text should be reworded, "Other studies are

17   supportive of this statement. For example, the statistical power of detection of this

18   study design supports this statement."

19

20   m)   *Pages 9-44, Table 9-19:* A footnote should be added to describe what the Z

21   statistic is and how Q O-2 are obtained. This section was very unclear.

22

23   n)   *Pages 9-40 thru 9-44, Section 9.3.4, pp. 5-3 thru 5.5 and Section 5.1.4*: The

24   Agency needs to address more fully the statistical and biological significance of the

25   testicular atrophy. The footnote on page 5-4, Table 5-1 states that statistics were

26   not conducted on the testicular lesions yet in Section 9.3.4 this endpoint becomes a

27   study for modeling. Is the background rate for this lesion in the   control B6C3 F1

1   mice low compared to historical mouse population statistics?

2

3   o)   *Pages 9-46, line 1-2:* In this example, the text provides a reason for discarding the

4   top doses in the modeling however, this decision is inconsistently applied in the

5   modeling Section 9.3 as evidenced in Table 9-16. The text should provide some

6   common guidance on what will be done regarding dropping higher dose levels

7   from modeling calculations.

8

9   p)   *Pages 9-46, line 10-11:* Statement in lines 10-11 appear to differ from more

10   recent research published on PBPK models. Can this health risk assessment go

11   further in using the data about known ovotoxicity of the diepoxide? See examples

12   provided in testimony from the Chemical Manufacturers Associations's documents.

13   (CMA, 1998 a,b)

14

15   q)   *Pages 9-51, lines 2-4:* The Committee was unconvinced about the superior nature

16   of the time-to-response modeling that was conducted for the LEC_{10} determination

17   for atrophy. Additional discussion is needed to support this statement especially

18   given the limitations of the biological time-to-response data for this endpoint.

19

20   r)   *Pages 9-51, lines 16-26:* The EHC agrees with these limitations but would then

21   use these points to justify using PBPK modeling to improve the target organ and

22   time concentration curves relative to these specific reproductive versus

23   developmental endpoints. The text has provided the justification but the

24   assessment falls short of acting on these suggestions.

25

26   s)   *Pages 9-51, lines 27-31:* The Committee agrees on these issues. Please see the

27   earlier comments on how to constructively address these points.

1

2   t)   *Mathematical errors need to be corrected.*

3   It is absolutely necessary that all calculations in Section 9 be carefully reviewed.

4   The Committee noted numerous problems in this section and refer USEPA to the

5   written testimony of R. Seilken that is especially relevant to this issue (Seilken, R.,

6   1998). There may be confusion with some numbers presented as natural logs

7   versus non-log numbers. Please proof very carefully. All calculations must be easy

8   to follow by a general scientific audience. Chapter 9 did not meet this goal.

9

10

11   **3.1.10 Chapter 10 - Weight of Evidence**

12

13   *Cancer endpoint*

14

15   There was a range of opinion on the committee about whether one can conclude that 1,3-

16   butadiene is a known human carcinogen. There was consensus on the Committee that there is

17   sufficient evidence to say that working in synthetic rubber production is causally associated with

18   leukemia. However, the majority felt that there were conflicting results among SBR and monomer

19   workers for leukemia and a lack of compelling evidence for a relationship between

20   lymphosarcoma and butadiene exposure. There was concern that an increased risk of leukemia

21   was not seen in the epidemiology studies of workers in the butadiene monomer industry. To some

22   EHC members, this lack of association significantly reduced confidence in the assumption that

23   1,3-butadiene was the causative agent for leukemia in the SBR industry study. In addition,

24   confounding or coexposure to other chemicals could not be ruled out with confidence.

1

2   There were a variety of views amongst the EHC as to how the call should be made on

3   whether a substance can be considered a "known human carcinogen." One view was that the

4   human data - from observational studies of cancer in humans - must stand on its own to make the

5   finding of "known human carcinogen", without regard to mechanistic or other information. A

6   second view was that the cumulative evidence, from human and animal studies, as well as

7   mechanistic data, particularly as it relates to human findings, should be used as the basis for the

8   judgment. The majority of the Committee felt that the judgment should be made on the basis of

9   human cancer observations alone and the evidence was not sufficient for 1,3-butadiene. A few of

10   the Committee members considered the human evidence in and of itself sufficient or that the

11   cumulative evidence was sufficient to make a finding of "known human carcinogen." Other

12   Committee members considered the body of mechanistic data to be indicative of the fact that

13   significant interspecies differences in response to 1,3-butadiene exist between rodents and

14   humans. For these members, the mechanistic data were consistent with 1,3-butadiene not being

15   classified as a known human carcinogen.

16

17   With respect to the narrative discussion in the evaluation, the Committee felt that it should

18   more reflect the range of opinion on the matter when discussing the human findings. Clearly

19   1,3-butadiene, when mutagenic or clastogenic, is so through its metabolism. This finding should

20   be provided in the evaluation, as well as a statement regarding the mechanistic studies most

21   relevant to humans.

22

23   *Non-cancer endpoints*

24

25   The weight of evidence is confined to addressing cancer endpoints. The reproductive

26   endpoint is the basis for RfC calculations and, therefore, also should be addressed.

27

1   **3.1.11 Chapter 11 - Risk Characterization**

2

3   The majority of the Environmental Health Committee did not classify 1,3-butadiene as a

4   known human carcinogen due to the lack of consistency between exposure and leukemia or

5   lymphosarcoma data when the styrene-butadiene rubber (SBR) and monomer worker studies were

6   considered in total. The majority opined that 1,3-butadiene should be classified as a probable

7   human carcinogen where as a minority felt that the science supported the classification of

8   1,3-butadiene as a known human carcinogen. The vast majority of the panel felt that the SBR

9   process supported classification as a known carcinogen.

10

11   The majority of the EHC was reluctant to classify 1,3-butadiene as a known human

12   carcinogen because it was felt that there was no consistent relationship between exposure and

13   leukemia or lymphosarcoma when the SBR and monomer studies were considered in total. Only

14   one study population (SBR industry) had credible leukemia excess related to exposure. Leukemia

15   was not elevated or related to estimated exposure in butadiene monomer industry.

16   Lymphosarcoma was only elevated in short term, not long term workers in the monomer industry

17   and also not elevated in the SBR industry. Hence, there is no dose response for the

18   lymphosarcoma. The lymphopoietic cancers should be considered separately when assessing

19   consistency across studies. The majority of the Committee felt that the finding of "known human

20   carcinogen" should solely be based on observational studies in humans, without regard to

21   mechanistic or other information. Others on the Committee felt that 1,3-butadiene should be

22   identified as a "known human carcinogen" because the cumulative evidence, from epidemiology,

23   animal cancer bioassays, and mechanistic studies should be used as the basis for the judgment.

24

25   In substantiating EPA's classification of 1,3-butadiene as a carcinogen, it is important that

26   the following points be made so that the strengths and weaknesses of the data are discussed.

27   a)   *Page 11-1:* The statement regarding the lack of sufficient data to determine if

1   children or other subpopulations are affected differently by exposure to

2   1,3-butadiene should be revisited in light of the studies by Nelson et al., 1995,

3   Wiencke, et al, 1995. Nelson et al., 1995 found the glutathione transferase theta

4   (GSTT1) to be highly polymorphic wide variation in its ethnic distribution (Nelson

5   et al., 1995). Wiencke et al., 1995 have shown the association between the

6   genotype for the production of glutathione transferase theta and genetic anomalies

7   in sister-chromatidic exchange induced by metabolites of 1,3- butadiene:

8   epoxybutene and diepoxybutane. This implies that the protein produced by the

9   gene is important in conjugating both of these metabolites. GSTT1 only affects

10   DEB and GSTM1 only affects EB. These are two proteins from two genes. Given

11   the profound racial distribution of the polymorphism, it is important to note that

12   this may account again for the significant portion of the alterations in the

13   metabolism of 1,3-butadiene. Consequently, the enzymes responsibilities for the

14   metabolic conversion of 1,3-butadiene to its mono- and di- epoxide forms as well

15   as its diol form are highly polymorphic. This implies that there may be differential

16   susceptibility to the genotoxic effects of exposure to butadiene. Investigations of

17   this are rapidly moving ahead. There is some indication from field studies that

18   these polymorphisms may contribute to directly- measurable genetic effects (Sorsa

19   et al). Hence, risk assessment and future studies of this compound should take note

20   of this and adjust as is appropriate.

21   b)   *Page 11-3, line 11-13 and Table 11-1*: EPA states that the conclusion of

22   "sufficient evidence" of human carcinogenicity is based on more than 10

23   epidemiologic studies examining five different groups of workers. This statement is

24   misleading because it implies that there is a consistency of results across several

25   studies of equal caliber examining completely different populations. The

26   predominant emphasis should be on the methods and findings of the two latest

27   studies of butadiene monomer and SBR workers. The emphasis on the early

1   epidemiologic studies is misleading to the extent that it gives the impression of

2   consistency across several study populations in the SBR and butadiene monomer

3   industries. There is really only one study population in each industry that provides

4   appreciable information. Each of these studies consolidated and/or more accurately

5   refined the populations used in the earlier studies. This needs to be clarified

6   because it is central to the understanding of how consistent the relationship is

7   between 1,3 butadiene and cancer. For example, EPA should explain that the

8   Delzell study included all of the eligible population from the Matanoski (1990)

9   study and the Meinhardt study (1982). The leukemia finding in the Matanoski et al.

10   (1990) study should not be presented as if it were a separate finding in a

11   completely different population from the Delzell study. In addition, the Delzell

12   study supersedes this and other previous studies and rectifies many of the

13   limitations and errors of the earlier studies.

14

15   c)   *Page 11-5, line 12-34:* The Matanoski study is presented as if it were a study on a

16   completely different population from the Delzell study. In discussing the excess

17   leukemia risk in the nested case-control study in the Matonoski study, it is

18   important to indicate in line 14 that there was no excess of leukemia observed in

19   the cohort study (standardized mortality ratio was 1.0 representing 22 observed,

20   22.9 expected). This is an important major point to be made up-front in this

21   section because it helps explain the scientific debate later referred to in lines 26-

22   28. The evidence linking butadiene exposure and cancer is still strongest for

23   leukemia based on one large, high quality cohort study of SBR workers (Delzell

24   studies) which supercedes the Matanoski study.

25   d)   *Pages 11-6 to 11-7:* It is never clearly stated that the leukemia excess seen in the

26   Delzell study has not been replicated in a completely different study population.

27   The studies of butadiene monomer workers and of other butadiene exposed

1   workers report null results for leukemia.

2   e)   *Page 11-7, line 17:* The lymphosarcomas and leukemias are discussed as if they

3   could be considered as the same type of cancer. It is implied that two different

4   populations have an excess in lymphohematopoietic cancers thereby demonstrating

5   consistency across studies. The strength of association evaluation should focus on

6   these different cancers separately.

7   The EPA review states on page 11-7, lines 20-26 that the monomer workers

8   exposed to shorter periods of time probably had higher exposures than workers

9   exposed for longer periods of time. This statement needs to be removed as there is

10   no evidence for this. The available evidence is insufficient for a causal relationship

11   between butadiene and lymphosarcoma. Lymphosarcoma was elevated for short

12   term exposed workers but not among long term exposed workers indicating a lack

13   of dose-response. Of equal importance, lymphosarcoma/non-Hodgkins lymphoma

14   (NHL) was not elevated in the SBR cohort which had excess leukemia. These

15   points must be discussed in the EPA document. The possibility that it is the SBR

16   process and not butadiene alone that may explain excess leukemia should be

17   discussed regardless of final decision on cancer classification. Delzell's finding that

18   the leukemia excess concentrated among workers who began employment in the

19   1950's and not those that worked exclusively in 1940's led to hypothesis by Irons

20   and Pyatt (1998) that DMDTC might be a contributing factor in the leukemia

21   excess. This hypothesis needs to be discussed as it is part of the scientific

22   literature.

23   f)   *Page 11-8, Table 11-2*: Some of the entries in this table seem unbalanced.

24   i)   Matanoski is cited as showing "7 to 9 times higher relative odds for

25   leukemia" without mentioning that others analyzed the same data using a

26   slightly different cutpoint and found an odds ratio of <1.0.

27   ii)   Most of the dose-response tabulations by Delzell et al. 1996 do not show

1   mortality ratios in the highest dose groups as high as the ones cited.

2   iii)   It states that a dose-response relation for monomer plant workers "Cannot

3   be demonstrated due to lack of quantitative exposure data," when in fact

4   several dose-response analyses were performed (albeit with an imperfect

5   dose metric) and showed not even a hint of an association.

6   g)   *Page 11-9, Line 24*: The Committee classified the SBR process as a known human

7   carcinogen.

8   h)   *Section 11.3.3, Page 11-9*: This section needs to more accurately reflect the

9   scientific literature that demonstrates a clear difference in metabolic activation

10   between humans and rodents and should discuss how PBPK modeling can refine

11   the risk assessment process. If EPA cannot incorporate PBPK modeling into risk

12   assessment within the mandated time-contraints, EPA should discuss future

13   possible directions and consider alternative ways (reduced safety factor) to account

14   for the species differences.

15   i)   *Page 11-10, line 36 to Page 11-11, lines 1-2*: The EPA states that the tumor type

16   in rodents most analogous to the lymphohematopoietic cancers is the lymphocytic

17   lymphomas. To properly discuss the strengths and weaknesses of this statement,

18   EPA should discuss the data generated by Irons et al, 1996 which shows that T-

19   cell lymphoma in mice is due to a specific population of stem cells in the mouse

20   bone marrow that is not present for humans or rat bone marrow cells. EPA should

21   also point out that the link between 1,3-butadiene exposure and lymphoma is weak

22   as there was no consistent dose-response relationship. Additionally, the Agency

23   should show consistency in combining different tumor types across documents. For

24   example, in the revised cancer risk assessment guidelines a case study for an

25   aromatic hydrocarbon (presumably benzene) is presented where mice are shown to

26   develop lymphomas following exposure whereas humans develop acute

27   myelogenous leukemia. In this case, the Agency did make the distinction that the

1   response in the animal models and the response in the humans was different. The

2   Agency should acknowledge that there is considerable disagreement as to whether

3   these two tumor types, lymphomas and leukemias, derive from the same origin.

4   The potential impact of task specific peak exposures must be addressed. The

5   primary metric for SBR workers is based on time weighted average exposure. But

6   SBR workers frequently get the majority of their exposures during a small fraction

7   of the work day during the conduct of specific tasks. The leukemogenic effect was

8   associated with those jobs that involved high peak exposures in latex sampling

9   (laboratory workers and in vessel cleaning for maintenance laborers). The general

10   population is typically exposed to lower ambient levels. An additional uncertainty

11   that should be discussed is the uncertainty of extrapolating from an occupational

12   setting where peak exposures occurred to the generally low ambient levels of

13   exposure.

14   j)   *Page 11, Lines 11-13*: The statement that the evidence regarding human

15   carcinogenicity is based on ten studies is deceptive, because the reports are not

16   independent, but most reports are updates of previous ones. In addition, there are

17   basically only four independent cohorts, not five, because the Delzell study

18   included about 95% of the workers in the Matanoski studies.

19   k)   *Section 11.5, Pages 11-13-11-14:* This section needs to be re-evaluated in light of

20   the two more recent negative dominant lethal studies. A weight of evidence

21   approach should be taken looking at all 3 studies rather than to emphasize the one

22   positive study.

23   l)   *Section 11.5 Pages 11-14, lines 1-3:* This sentence should be rewritten as fact not

24   as speculation.

25   m)   *Pages 11-14, lines 10-16:* These two sentences should be rewritten or removed.

26   Text discusses concept of "meaningful increases in risk." What is the definition of

27   this phrase? Is this in the USEPA guidelines? This phrase appears to be an

1   editorial. For this reviewer to understand that "...effects are not expected in

2   humans exposed to low environmental exposures. . . " the assessment must provide

3   a detailed rationale that follows from Chapters 5 and 9. This summary statement

4   was not supported in the document.

5   n)   *Section 11.6, Pages 11-15*: This summary section needs to be integrated with the

6   biology discussed in Chapter 5 and quantitative assessments in Chapter 9. No new

7   "surprise," non-justified statements should appear in this section.

8   o)   *Section 11.6*: The importance of the positive heritable translocation studies in mice

9   should be more heavily emphasized.

10   p)   *Section 11.6, Page 11-15, line 10-17*: The additional safety factor of 3 to

11   extrapolate from a LOAEL to NOAEL is inappropriate and should be removed.

12   Inadequate justification is given for the application of the additional safety factor

13   for the benchmark dose. The EHC could not understand the rationale for its

14   inclusion. This section must discuss the rationale for using the "hybrid model" for

15   continuous data analysis and exactly what the hybrid model is so that the risk

16   assessment can be completely transparent. On the surface it appears that the

17   approach yielding the lowest LEC was selected. Some of the EHC

18   Members/Consultants recommend that the Agency use the EC10 (central estimate)

19   as the point of departure rather than the LEC10. Other EHC Members/Consultants

20   did not agree with the recommendation.

21   q)   *Section 11.8 on Page 11.16*

22   The Agency should expand on future research needs to fill gaps in knowledge. The

23   list is inadequate.

24

25   **3.2   Classification of 1,3-Butadiene as a Known, Human Carcinogen**

26   *Does the science support the classification of "known" human carcinogen?*

27   There was a majority opinion amongst the Committee that the science supports the SBR

1   process as a known human carcinogen, not butadiene monomer. The lack of positive findings

2   regarding lymphosarcoma in the large Delzell cohort and the lack of positive findings regarding

3   leukemia in the three butadiene monomer studies weaken the case for a straightforward causal

4   association between these endpoints and 1,3-butadiene exposure. The EPA report suggests these

5   discrepancies may be due to: 1) butadiene "dose rate," 2) different confounding factors, or 3)

6   different "co/modifying" factors (Page 11-7). The first reason seems very implausible. There is no

7   known example of a chemical or substance that causes different cancers depending on dose rate,

8   especially at the relatively low dose rates in these studies (unlike the rodent studies with hundreds

9   of ppm). The second reason raises the possibility that one or both of the putative associations may

10   not be real but may be caused by confounding by some unknown factor(s). This reason weakens

11   the case for causality. The third reason, that there may be some cofactor or modifying factor,

12   suggests that even though butadiene might induce cancer in the presence of some cofactor or

13   modifying factor, it would not do so in the absence of that factor. If this is the case, then it would

14   be inappropriate to generalize to other exposure scenarios for the general public since they would

15   likely not have concomitant exposure to the cofactor. There is some evidence to support this later

16   point in the studies of Leavens et al. (1997) in which mice were exposed to mixtures of

17   1,3-butadiene and styrene as well as 1,3-butadiene or styrene alone. Genotoxicity was noted in

18   mice exposed to 1,3-butadiene; cytotoxicity was noted in mice exposed to styrene; both

19   cytotoxicity and genotoxicity were noted in mice exposed to the mixture. This study provides

20   some mechanistic support for the observation of leukemia in SBR workers but not in butadiene

21   monomer workers as it is consistent with biologically-based models that demonstrate the

22   requirement for both cytotoxic and genotoxic events for the development of cancer. In short, this

23   inconsistency in findings, whatever its basis, weakens the case for causal associations and/or that

24   such associations can be generalized to exposure of the public to butadiene.

25

26   The case for an association between lymphosarcoma and butadiene exposure is weakened by

27   the fact that in the main study (Divine, et al., 1996) that purportedly showed the association, there

1   was no indication of an exposure-response relationship nor was there evidence that those with

2   longer-term exposure had a higher risk of lymphosarcoma.

3   On page 11-3, lines 11-13, the report claims that "'sufficient evidence' of human

4   carcinogenicity is based on more than 10 epidemiological studies examining five different groups of

5   workers" and summarizes them in Table 11-1. But there are effectively only 4 groups of   workers,

6   not 5, since the Matanoski and Delzell cohorts have a high degree of overlap (i.e., about 95% of the

7   Matanoski cohort is included in the Delzell cohort). In addition, the "more than 10" studies are just

8   earlier reports of the same cohorts and do not add anything to an inference of causality beyond that

9   seen in the most recent follow-ups. Of the four independent studies, one   small one Cowles, et al.,

10   1994 is completely negative for lymphosarcoma and leukemia; another small one was positive for

11   lymphosarcoma, but based on only 4 cases, and showed no excess of leukemia (Ward et al., 1996); a

12   relatively large study was mostly negative, but a bit suggestive,   of a lymphosarcoma excess, but

13   provided no support for a leukemia excess (Divine, et al., 1996); and one large study was positive for

14   leukemia but not for lymphosarcoma (Delzell, et al., 1996) .

15   In summary, the weight of epidemiological evidence does not support an association

16   between butadiene exposure and lymphosarcoma/reticulosarcoma.

17   While the Delzell study (Delzell, et al., 1996, Macaluso, et al., 1996) is a large and

18   methodologically sound study, one would like to see at least a second independent confirmatory

19   study before affirming there is "sufficient evidence of human carcinogenicity" regarding butadiene

20   and leukemia. Instead, one sees a fairly large and reasonably sound study that shows no leukemia

21   excess (Divine, et al., 1996) plus two smaller ones with no evidence of leukemia risk, and these

22   weaken the case.

23

24   A majority of the EHC members felt that the body of mechanistic data on butadiene does

25   not support the classification of known human carcinogen, while others did not. The Agency

26   notes (page 9-52) that there are large unexplained differences in the response of rats and mice to

27   butadiene and states that the specific mechanisms of 1,3-butadiene induced carcinogenesis are

1   unknown. However, there is strong evidence from both metabolism and genetic toxicology studies

2   that diepoxybutane is a critical metabolite in the carcinogenic process. The extensive capability of

3   mice to form the diepoxide metabolite and the extensively higher levels of the diepoxide

4   metabolite in blood and tissues of mice compared with rats, most likely forms the basis for this

5   dramatic species difference in carcinogenic response. This is an important point because it

6   suggests that the diepoxide metabolite may be the best dosimeter for assessing risks for humans

7   exposed to butadiene.

8   Toxicokinetic modeling cannot prove or disprove the relationship between chemical

9   exposure and toxic or carcinogenic effect. However, these models can be developed to test

10   quantitative hypotheses regarding proposed mechanisms of toxicity or carcinogenicity of

11   chemicals. The Agency notes that "pharmacokinetic modeling of 1,3-butadiene has not elucidated

12   the reasons for interspecies differences in carcinogenic response between rats and mice" and "mice

13   and rats also exhibit substantial quantitative differences in their metabolism of 1,3-butadiene to

14   potentially reactive metabolites. Unfortunately, existing pharmacokinetic models have been unable

15   to explain the species differences in carcinogenic response." These statements are not true if one

16   considers the current PBPK models that describe the disposition of butadiene, epoxybutene and

17   diepoxybutane. These PBPK models clearly simulate the dramatic species differences in tissue and

18   blood concentrations of diepoxybutane between rats and mice observed in vivo. The models

19   suggest that species differences in response to butadiene are most likely related to differences in

20   rates of formation and removal of diepoxybutane. Experimental data and PBPK model simulations

21   indicate that mice produce far greater concentrations of this reactive metabolite compared with

22   rats.

23   These PBPK models can be, and have been, extended to humans. In vitro data on rates of

24   butadiene, epoxybutene, and diepoxybutane metabolism obtained in human tissue samples can

25   also be used to predict blood and tissue concentrations of both epoxide metabolites in humans.

26   When these simulations are conducted using average values for human metabolic rates results

27   indicate that human diepoxide concentrations would be orders of magnitude less than those of

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1   mice and lower but much more similar to concentrations predicted for rats. Nonetheless,

2   1,3-butadiene produces cancer in rats, albeit at higher dose levels than for mice, so similarity of

3   human and rat metabolism would still contribute to the weight of evidence. Some Committee

4   members point out limitations in the models and the underlying assumptions regarding choice of

5   human parameters and activity of metabolites. These members also point to the considerable other

6   mechanistic data (which are described in comments on Chapter 4).

7

8   **3.3   Approaches Taken to Characterize Plausible Cancer Risks**

9   *Are the approaches taken to characterize plausible cancer risks reasonable given the science?*

10   Butadiene is one of the first chemicals to be brought forward under the EPA's new risk

11   assessment guidelines. It needs to be a model of how to proceed. The Agency must clearly

12   separate known from unknown and important from unimportant. The database for butadiene is

13   very challenging and robust, but all of the important issues are not settled. The EPA must state

14   the facts in proper perspective and should not be afraid to say that more information is needed.

15

16   Risk estimates use external butadiene exposures as the dose estimate given the

17   sophisticated PBPK models currently developed and the availability of in vitro metabolic

18   parameters in rodents and humans an approach that used an estimate of internal dose . The

19   Agency notes (Page 11-12, Lines 1-3) that a review of the available pharmacokinetic data and

20   models reveal that the state of this science is currently inadequate for either explaining

21   interspecies differences or improving on default dosimetry assumptions. As noted previously in

22   this report, this statement should be revised to reflect the available scientific data and

23   pharmacokinetic models. The fact that human variability in metabolic response is noted should not

90

1   be viewed as a limitation for these models, but instead as an opportunity to reduce uncertainty and

2   characterize variability. If metabolic activation of butadiene is necessary to produce genetic

3   damage, and ultimately carcinogenicity, which is not an unreasonable hypothesis, then individuals

4   with the highest metabolic capacity for activation and the lowest metabolic capacity for

5   deactivation would be at most risk for exposure to butadiene. The PBPK model could be used to

6   determine the extent to which metabolic differences among humans is the dominant process

7   controlling the magnitude of the effective dose at the target site.

8   The Agency notes (page 9-16, lines 1-7) that risk assessments based on rat carcinogenicity

9   data "are not considered the most appropriate estimates of human risks .... EPA believes that the

10 mouse is likely to represent a better rodent model for human cancer risk assessment from

11 1,3-butadiene." Available mechanistic data on the formation of epoxybutene and diepoxybutane

12 obtained in rat, mouse and human tissue samples suggest that the rat is a more appropriate model

13 for assessing risks for humans than is the mouse. Choosing a rat versus a mouse as the most

14 appropriate animal for assessing risks in humans may, in fact, be an oversimplification. As noted

15 above, the development of PBPK models that are capable of predicting concentrations of the

16 reactive metabolites in the target tissues and availability of distributions of human metabolic rate

17 constants would allow one to not rely on either mouse or rat per se. Instead one can use all of the

18 available mechanistic data within the context of a PBPK model to predict the butadiene doses in

19 humans necessary to yield epoxide concentrations in tissues similar to those predicted for rats or

20 mice exposed to carcinogenic concentrations of butadiene.

21   The Agency notes (page 9-17, lines 6-11) that no attempt was made to adjust for internal

22 doses of reactive 1,3-butadiene metabolites because the PBPK data were inadequate to develop a

23 reliable PBPK model. As noted previously PBPK models not reviewed by the Agency in this   draft

24 document have been developed that are capable of predicting the butadiene epoxybutene   and

25 diepoxybutane blood and tissue concentrations in rats, mice and humans following exposure   to

26 butadiene. In this case, a human PBPK model could be readily used to obtain either point estimates

27 using average human values or a range of estimates by conducting Monte Carlo simulations to

1   sample from a distribution of available parameter values for humans for both metabolic rate

2   parameters and physiological parameters. A plausible assumption is that for a   given diepoxybutane

3   tissue concentration the human response would be equivalent to the rodent response. Then,

4   concentrations of butadiene necessary to elicit tissue doses of diepoxybutane in humans equivalent to

5   diepoxybutane concentrations in mice or rats at doses which yielded   tumors could be calculated.

6   PBPK models of these and the diepoxybutane and other active metabolites can then be applied to

7   human risks predictions. The advantage of using a PBPK   model is that mechanisms underlying

8   absorption, distribution, metabolism and elimination of butadiene, epoxybutene, epoxybutanediol and

9   diepoxybutane which are either similar or different across species and across doses would be

10   accounted for. Given the availability of these PBPK models, this approach would be far superior to

11   using a simple arithmetic adjustment for   continuous daily exposure versus exposure under bioassay

12   conditions.

13   On page 9-25, lines 1-7 the EPA notes that "mice and rats also exhibit substantial quantitative

14   differences in their metabolism of 1,3-butadiene to potentially reactive metabolites. Unfortunately,

15   existing pharmacokinetic models have been unable to explain the species   differences in

16   carcinogenic response." A majority of the Committee assert that there are several published PBPK

17   models that have been able to successfully explain species differences in the carcinogenic response

18   exposure to 1,3-butadiene based on differences in the metabolism of 1,3-butadiene to reactive

19   metabolites while other Committee members were concerned about the degree to which some

20   models were able to describe the available data, the extent to which parameters had to be changed

21   from measured values to obtain reasonable fits and the accuracy of the low dose extrapolation. In

22   particular, the highly mutagenic diepoxybutane is formed to a   much greater extent in mouse

23   tissues compared with rats.

24   Again on page 9-25, lines 15-19, the EPA notes that "ideally a PBPK model for the   internal

25   dose of the reactive metabolites would decrease some of the quantitative uncertainty in interspecies

26   extrapolation. However, current PBPK models are inadequate for this purpose." The available

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1   pharmacokinetic models and emerging data on human pharmacokinetics should be   further explored

2   for use in risk assessment. As noted in the previous comments, some of the Committee members

3   assert that PBPK models that are capable of interspecies and low dose extrapolation for this purpose

4   do exist. Other Committee members assert that although the   PBPK models require validation and

5   development they may prove useful in the near term and   that models should be further developed

6   to address parameter and structural uncertainty as well   as interindividual variability in human

7   metabolism, but the tools for such activity are at hand.   On page 9-51 the Agency notes that

8   NOAEL LOAEL EC10 or LEC10 should be converted to appropriate human equivalent exposures

9   before using these exposure levels as points of departure and that theoretically this is best

10 accomplished using a PBPK model. The Agency also notes that the current PBPK models are

11 inadequate for use in risk assessment. However, as noted   previously, some of the Members felt

12 that there are several available PBPK models that are   capable of predicting diepoxide

13 concentrations in target tissues for rats, mice and humans and   that any one of these models could

14 be used to obtain a more appropriate human equivalent exposure. Other Committee members felt

15 that, at a minimum, the different toxicokinetic   hypotheses and the hypothesized role of the various

16 metabolites should be discussed, at least qualitatively, with an indication of the degree to which the

17 assessment would be impacted if   some of the hypotheses were later proven true. In the

18 1,3-butadiene health risk assessment, the Agency should address the variety of viewpoints and

19 hypotheses of research in the field of PBPK modelling.

20   The EPA report pays relatively little attention to the issue of how much peak exposures to

21 butadiene may have influenced leukemia risk in the Delzell study. It indicates (P. 7-22, L.   30-33)

22 that there was an association of peak exposures (defined as >100 ppm) with leukemia but dismisses it

23 as an "irregular" association. The Acquavella commentary on the EPA draft that   was sent to the

24 EHC (Acquavella, 1998) presents a table showing how much peak exposures   affect the

25 association of butadiene ppm-years with leukemia risk (Page 10). The formal linear regression

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1   estimates with and without adjustment for peak exposures are not presented, but it is apparent that

2   the regression estimate would be appreciably less when peak exposure was adjusted for. Specifically,

3   for the three highest cumulative exposure groups, the excess relative risks with and without control

4   for peak exposures were 0.0 and 1.0 respectively for 20-99 ppm-years, 0.3   and 1.4 respectively

5   for 100-199 ppm-years, and 1.5 and 3.6 respectively for 200+ ppm-years. In each dose group,

6   adjustment for peak exposures reduced the leukemia risk substantially. Since butadiene exposures to

7   the public will almost never approach the peak exposure range, a more appropriate model for risk

8   would factor out the peak-exposure component.

9   On Page 9-2, Lines 24-25 regarding the Delzell analysis of butadiene exposure vs.

10   leukemia, it is noted that "excluding exposures within 20 years of death weakened and almost

11   eliminated the relationship...." This indicates that in modeling lifetime risk, a model that assumes

12   a limited effect time (i.e., that leukemia risk during a given year of age is affected largely by the

13   butadiene exposures received during the previous, say, 20 years, and only slightly or not at all by

14   more distant ones) should be considered. This "windows of exposure" model has precedents, e.g.,

15   lung cancer risk from radon has been modeled in this way in a National Academy of Sciences

16   report (NAS/NRC, 1988) because lung cancer risk was little affected by radon exposures in the

17   distant past; leukemia risk from radiation is highly elevated at 5-10 years after irradiation but there

18   is little elevation by 20-30 years after irradiation (NAS/NRC, 1990). If this model were

19   considered for projecting lifetime risk, it would show appreciably less risk from chronic exposures

20   than does the present one, which assumes that excess relative risk at, say, age 70 is an additive

21   function of all the exposure accumulated in the previous 69 years.

22   **3.4   Conclusions and Quantitative Estimations for Reproductive/Developmental**

23   **Effects**

24   *Are the conclusions and quantitative estimations for reproductive/developmental effects*

25   *adequately supported?*

26   The Committee supports the Agency's use of benchmark dose procedures and the

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1   modeling of reproductive toxicity endpoints. The EHC also supports the continuing attempts to

2   develop new strategies, such as those presented in the report to address ovarian, uterine and

3   testicular atrophy, to quantitatively address reproductive endpoints in risk assessment documents.

4   This is one of the first such assessments and some general suggestions are made regarding   conduct

5   of the analyses and the presentation of results for the analyses:

6   a)   A clear rationale for the selection of one particular point of departure over another   is

7   needed -- for each case for which the default is applied and for cases deviating from

8   the default.

9   b)   Where it is not obvious the biological significance of particular endpoints for the

10   assessment should be conveyed.

11   c)   When applying novel approaches, such as the time-to-response modeling of

12   atrophy, the advantages and limitations in utilizing the approach in the context of

13   reference concentration estimation should be clearly understood and presented.

14   d)   The incorporation of information on toxicokinetics (e.g., ovotoxicity of diepoxide)

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1   should be used where possible on at least a qualitative if not quantitative basis to

2   inform the choice of endpoints and modeling.

3   e)   The exclusion of points at high doses contributing to a poor fit is a reasonable

4   approach, and typically can be explained, for example, in terms of toxicities other

5   than the one being modeled, or toxicokinetics. When the approach is applied where

6   possible the specific rationale for the case at hand should be presented. The use of

7   the procedure and the rationale provided for should be consistent across endpoints

8   and across assessments for the same scenarios.

9   f)   There may be a difference of opinion among Committee members about the

10   appropriateness of using a risk reduction factor, when reference concentrations are

11   based on benchmarks associated with risk of effect. The rationale for use of this factor

12   needs to be clearly laid out.

13   g)   Analyses of this type applied to these endpoints may be unfamiliar to others

14   working in the general area. Confusion over the presentation of some results

15   (e.g., logarithm versus linear scales) was obvious in some of the comments

16   received. Also, calculations should be easily followed, and of course need to be

17   carefully proofed by someone other than the one making them. Tables and   figures

18   should be free standing, with statistics referenced.

19   ***Some of the Committee members are of the opinion that*** the quantitative estimations for

20   reproductive/developmental effects could benefit greatly from the application of PBPK modeling to

21   estimate the effective dose at the target site. The Agency notes (Page 9-46, lines 6-11) that

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1   "ovarian atrophy has been shown to be related to the amount of the diepoxide metabolite in the

2   tissue. Modeling of the ovarian atrophy and uterine atrophy data was considered based on   internal

3   dose of the diepoxide metabolite, however an adequate model was not available to   estimate levels

4   of the diepoxybutane." There are several PBPK models that are currently   published that allow

5   calculation of the dose of the epoxide metabolite in target tissue. Any one   of these models could

6   be used to determine the internal dose of the diepoxide metabolite and provide a more refined

7   estimate of risks for this reproductive endpoint.

8   # 4. SUMMARY OF RECOMMENDATIONS

9   a)   The EHC recommends that the Agency's updated, draft health risk assessment of

10   1,3-butadiene reflect the new information that has been published in the peer-   reviewed

11   literature since the Agency's cut-off date of January 31, 1997. The   inclusion of this

12   new information would greatly improve the technical quality and comprehensiveness of

13   the report.

14   b)   Since critical research like the Delzell et al. (1995) exposure reestimation and the

15   pharmacokinetic modeling has not been completed yet, the Committee   recommends

16   that the Agency's risk assessment of 1,3-butadiene be labeled as an   interim risk

17   assessment.

18   c)   The majority of the Committee recommends that the Agency classify 1,3-butadiene as a

19   probable human carcinogen because there was not consistency between the exposure and

20   leukemia or lymphosarcoma data when the SBR and monomer studies were considered in

21   total. Also, the role of confounders and cofactors was unclear.

22   d)   The Committee found the approaches taken to characterize plausible cancer risks to be

23   reasonable but points out specific improvements that can be made.

24   e)   The Committee supports the Agency's use of benchmark dose procedures and the

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1   development of mathematical models for reproductive endpoints. To improve the analysis

2   and the clarity of the results specific recommendations focused on:

1   (i)   providing a clear rationale for selecting one particular point of departure

2   over another,

3   (ii)   conveying the biological significance of particular endpoints of the

4   assessment,

5   (iii)   presenting the advantages and limitations of novel approaches such as the

6   time-to-response modeling of atrophy,

7   (iv)   incorporating information on toxicokinetics on at least a qualitative, if not

8   quantitative basis to inform the choice of endpoints,

9   (v)   excluding points at high doses that contribute to a poor fit,

10   (vi)   explaining the rationale for using a risk reduction factor, and

11   (vii) proofing calculations and correcting them where needed.

12   f)   In Chapter 1, Introduction, the Agency should state whether the different cancer

13   classification systems and quantitative assessments are equally valid and

14   scientifically defensible and explain the rationale. In addition, the Agency should

15   comment on its Cancer Risk Assessment Guidelines.

16   g)   In Chapter 2, Overview of Exposure to 1,3-Butadiene, the Agency should clearly

17   indicate that the chapter is not intended to be a comprehensive review of   exposure.

18   The chapter should include an explanation on how concentrations have been

19   measured over the years and how this might impact a comparison of   exposure

20   levels measured in earlier years with those from more recent years. Specific

21   recommendations for improvements for chapter 2 are included in Section 3.1.2.

1   h)   There are several recommendations regarding Chapter 3, Metabolism and

2   Pharmacokinetics. The main recommendations include the following with additional

3   comments included in Section 3.1.3.

4   i)   The title of Chapter 3, Metabolism and Pharmacokinetics, should be

5   changed to *Metabolism and Toxicokinetics* because 1,3-butadiene is not

6   used as a therapeutic agent.

7   ii)   The word, *toxicokinetics*, should replace the word, *pharmacokinetics*,

8   throughout the document.

9   iii)   Many new studies are not incorporated into the chapter. The Health

10   Canada assessment and the comments that were submitted by Dr.

11   Himmelstein provide many of those new references (Himmelstein, 1998).

12   iv)   The Agency should revisit its statement regarding species differences in

13   butadiene metabolism, taking into account the most recent information on

14   species differences in the production of diepoxybutane and other reactive

15   metabolites.

16   v)   The chapter should point out the close parallels between the observations   in

17   in vitro studies on metabolism and tissue concentrations of epoxybutene   and

18   diepoxybutane in mice and rats and in vivo studies on the metabolism of

19   butadiene.

20   i)   The Committee's recommendations for Chapter 4, Mutagenicity, are numerous.

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1   Some of the recommendations are given below and additional recommendations

2   are included in Section 3.1.4:

3   i)   The statement on Page 11-1 regarding the lack of sufficient data to

4   determine if children or other subpopulations are afffected differently by

5   exposure to 1,3-butadiene should be revisited in light of the studies by

6   Nelson et al., 1995, Wiencke et al. and Kelsey et al, 1995. Nelson et al.,

7   1995 found the glutathione transerase theta (GSTT1) to be highly

8   polymorphic wide variation in its ethnic distribution (Nelson et al., 1995).

9   ii)   Most of the extensive work on mutagenicity prior to 1994 should be

10   included.

11   iii) The chapter should include text tables that summarize key animal and

12   human findings derived from the entire body of information on butadiene.

13   iv)   The tables should include references to support key findings.

14   v)   There should be separate tables for in vitro, animal and human findings.

15   vi)   The similarities and species differences in response should be noted.

16   vii) The chapter should include more emphasis on the positive heritable

17   translocation studies in mice because of their potential relevance for human

18   heritable risks, and to several additional studies conducted in humans.

19   viii) The conclusion section should be expanded to include what is known about

20   the mutagenicity of 1,3-butadiene and its metabolites.

21   ix)   The missing dose units, missing units for mutant frequencies and other

1   similar omissions should be added.

2   x)   The explanation as to possible reasons for the discordance between the

3   positive effects obtained by autoradiography and the negative results found

4   by cloning for human 1,3-butadiene induced hprt mutations in vivo should

5   be rewritten.

6   xi)   The conclusion section should be expanded by adding statements

7   summarizing what is known about mutagenicity of 1,3-butadiene and its

8   metabolites.

9   j)   Some of the recommendations for Chapter 5, Reproductive and Developmental

10   Effects, are included in recommendations cited in (e). Additional recommendations

11   are provided in detail in Section 3.1.5 and are summarized below.

12   i)   All of the pertinent studies should be introduced in a summary table at the

13   beginning of each section.

14   ii)   Both positive and negative studies should be included so the reader can

15   develop a comprehensive understanding of the data base supporting the

16   assessment.

17   iii)   More current research should be included, especially the new dominant

18   lethal studies.

19   iv)   Chapter 5 should be integrated with the other chapters, especially the

1   chapters on pharmacokinetics and metabolism, animal toxicity, and

2   quantitative risk assessment.

3   v)   The Agency should identify where it is uncertain with regard to conclusions

4   about the reproductive and developmental endpoints, identify the action it

5   will take to respond to the uncertainty, and should include all of the

6   assumptions regarding uncertainty in the respective chapter.

7   k)   The Committee's recommendations on Chapter 6, Toxicity in Animals, are listed in

8   Section 3.1.6 and include the following:

9   i)   The rationale for the selection of the toxic non-cancer endpoint that is

10   utilized in the derivation of the RfC is very important and should be more explicitly

11   explained.

12   ii)   It is unclear whether all of the repeat dose studies have been reviewed by

13   the Agency. The EPA should incorporate the repeat dose in vivo

14   mammalian studies of 1,3-butadiene in Chapter 6 unless these are covered

15   elsewhere in the document and the Agency explicitedly states so.

16   iii)   The Agency should present the NTP study details in the first section

17   (chronic) of Chapter 6 and then refer to them in the carcinogenicity section.

18

19   iv)   The EPA should use the same categories of tumors for presenting data

20   from the continuous treatment, 9 and 15-month interim sacrifices and stop-

1   exposure study in order to permit comparisons.

2   v) A table summarizing the positive oncogenic findings across all studies

3   specifying the dose tested, and the type of tumor that was significantly

4   elevated, should be added since there were numerous organs and tumors

5   involved.

6   vi) The carcinogenic evidence on the mammalian metabolites of 1,3- butadiene

7   should be separated from the data on related chemicals and placed in a

8   subsection by itself.

9   vii) The rat carcinogenicity data should be presented in similar detail to the

10   NTP data so that the reader does not have to find the old risk assessment

11   to see the data.

12   viii) The observation that concentration, not time is a critical determinant of

13   potency is not supported by a comparison of the tumor data presented in

14   Table 6-4 and Table 6-8 from the NTP (1993) study for continuous lifetime

15   treatment and stop-exposure study.

16 l)   The Committee recommendations for Chapter 7 include:

17   i) A statement that, for the Delzell study (1996), there was no excess among

18   those hired before 1950 (Observed/Expected = 17/16.4 = 1.04) when one

19   would expect the highest exposures, but there was an excess among those

20   hired during 1950-59 (Observed/Expected = 20/10=2.0, Confidence

1   Interval = 1.2-3.1)

2   ii)   A statement regarding the inappropriateness of "lumping" the

3   lymphohematopoietic tumors should be added.

4   iii)   A statement regarding the possible role of confounding should be included

5   in the document.

6   m)   The recommendations for Chapter 8 are provided in Section 3.1.8 and make the following

7   revisions:

8   i)   The most recent relevant literature on butadiene toxicokinetic modeling

9   should be included.

10   ii)   The Agency should revisit its conclusion in Chapter 8 once it includes the

11   recent PBPK models as explained in Section 3.1.8.

12   n)   The recommendations for Chapter 9 are provided in Section 3.1.9 and include the

13   following:

14   i)   The 1997 data of Matanowski, et al. should be considered,

15   ii)   The Agency should explain or provide further explanation on the following:

16   a) why equivalent model fits for the Delzell data are obtained in order

17   to convey a greater appreciation of the nature of the dose response

18   data,

1   b)   the role of benzene as a confounder,

2   c)   the potential importance of the uncertainties of dose reconstruction

3   and the potential magnitude of the impact on the risk predictions,

4   d)   where possible, the rationale for the exclusion of points at high

5   doses contributing to a poor fit, and

6   e)   information on toxicokinetics (e.g., ovotoxicity of diepoxide).

7   o)   Chapter 10, Weight of Evidence, should be rewritten to reflect the range of

8   opinion regarding the human findings. In addition, the finding that 1,3-butadiene,

9   when mutagenic or clastogenic, is so through its metabolism should be provided in

10   both the evaluation and in the statement regarding the mechanistic studies that are

11   most relevant to humans.

12   p)   Specific recommendations for Chapter 11, Risk Characterization, are provided in

13   Section 3.1.11 and include the following:

14   i)   The statement that the conclusion of "sufficient evidence" of human

15   carcinogenicity is based on more than 10 epidemiologic studies

16   examining five different groups of workers should be rewritten

17   since it is misleading.

18   ii)   In discussing the excess leukemia risk in the nested case-control

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1   study in the Matanoski study, it is important to indicate that there

2   was no excess of leukemia observed in the cohort study.

3   iii)   The chapter should clearly state that the leukemia excess observed

4   in the Delzell study has not been replicated in a completely different

5   study population. In addition, a weight of evidence approach should

6   incorporate all three studies rather than to emphasize the one

7   positive study.

8   iv)   The strength of the association evaluation for the lymphosarcomas

9   and the leukemias should focus on these different cancers

10   separately.

11   v)   The EPA should incorporate the PBPK modeling into Chapter 11 if it

12   has time to do so or should at a minimum, discuss future possible

13   directions and consider alternative ways (e.g. reduced safety factor)

14   to account for the species differences.

15   vi)   Some members felt that the additional safety factor of 3 to move

16   from an effect dose to a no effect dose should be removed because

17   it is inappropriate.

18   vii)   The document should point out important data gaps in our

19   knowledge and research needs.

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18   * This document was reviewed by the Committee.
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1 ## APPENDIX A - ACRONYMS AND ABBREVIATIONS
2

3 4	ACGIH-TLV	-	American Conference of Governmental Industrial Hygienists - Threshold Limit Value
5	Bdiol	-	3-butene-1,2-diol
6	CI	-	confidence interval
7	DEB	-	1,2,3,4-diepoxybutane
8	DMDTC	-	Dimethyldithiocarbamate
9	EB	-	1,2 epoxy-3-butene
10	EBD	-	3,4-epoxy-1,2-butanediol
11	Ec_x	-	effective concentration at x% risk, ranging from 0.1% to 10%
12	EHC	-	Environmental Health Committee
13	ED	-	effective dose
14	EBdiol	-	1,2 dihydroxy-3-4 epoxybutane
15	GC-MS	-	gas chromatography - mass spectrometry
16	GSTT1	-	glutathione S-transferase theta
17	GSTM1	-	glutathione S-transferase μ
18	HEI	-	Health Effects Institute
19	ICD	-	International Classification of Diseases
20	JEM	-	job-exposure matrices
21	K_m	-	substrate concentration at one-half maximum velocity

1	LEC_p	-	95% lower confidence intervals associated with a risk (p), ranging from
2			1% to 10%
3			LOAEL - lowest-observed-adverse effect
4	MACT	-	maximum achievable control technology
5	mg	-	milligram
6	MLE	-	maximum likelihood estimate
7	NCI	-	National Cancer Institute
8	NHL	-	non-Hodgkins lymphoma
9	NOAEL	-	no-observed-adverse effect
10	NTP	-	National Toxicology Program
11	O/E	-	observed/expected
12	OMS	-	Office of Mobile Sources
13	ORD	-	Office of Research and Development
14	PBPK	-	physiologically-based pharmacokinetic
15	ppm	-	parts per million
16	ppmh	-	parts per million per hours
17	q_1	-	the incremental unit cancer risk for humans
18	RfC	-	Inhalation Reference Concentration
19	RR	-	relative risk
20	SBR	-	styrene butadiene rubber
21	SCE	-	sister chromatid exchange

1   SOT   -   Society of Toxicology

2   THB   -   trihydroxybutane

3   TRI   -   Toxics Release Inventory

4   UAB   -   University of Alabama at Birmingham

5   V_{mzx} -   maximum velocity for an enzyme-mediated reaction

6   WWII   -   World War II

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